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# A Photometric Method of Studying the Control of Handedness by the Cerebral Cortex of the Rat

Donald K. Gucker

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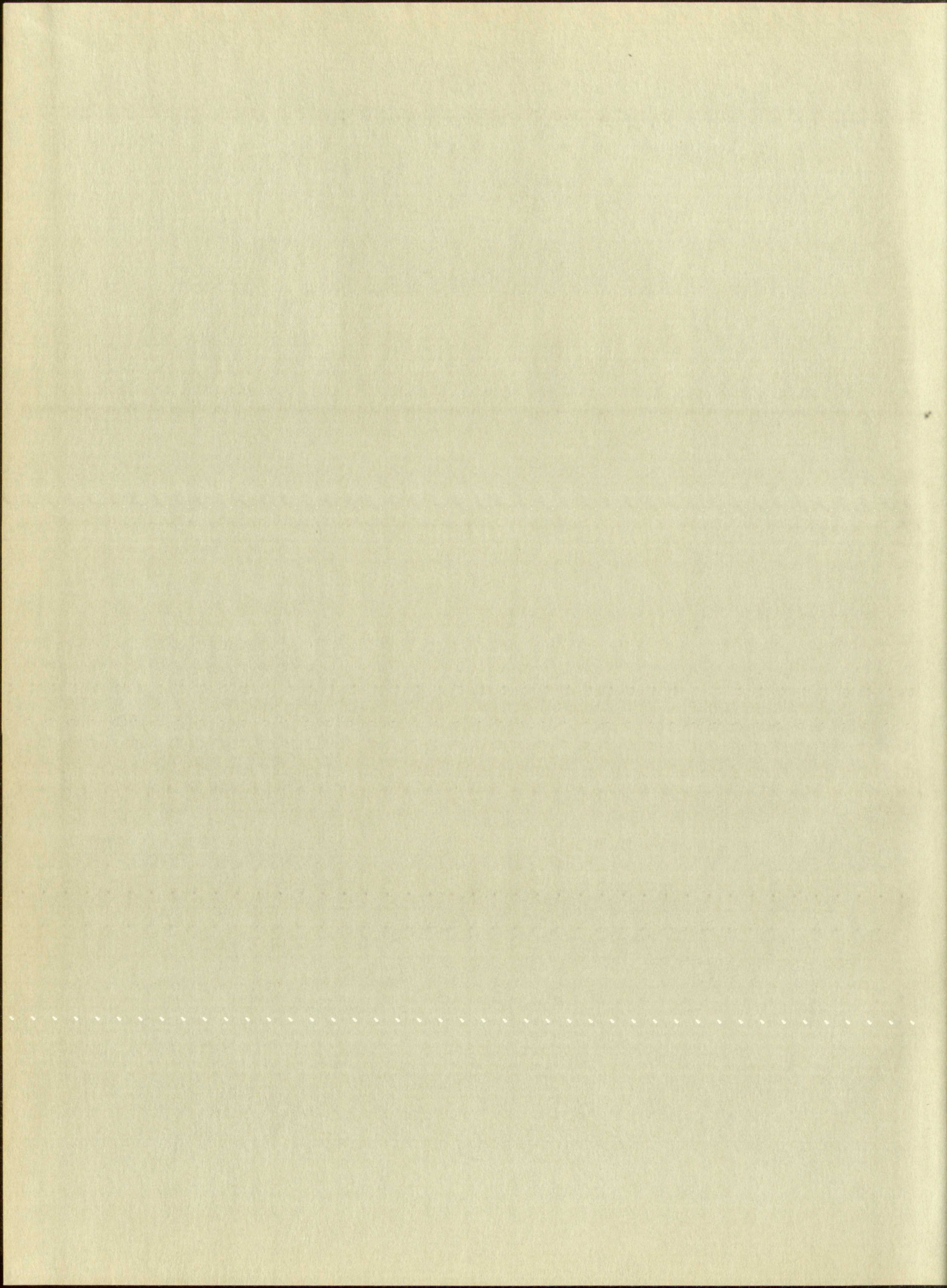
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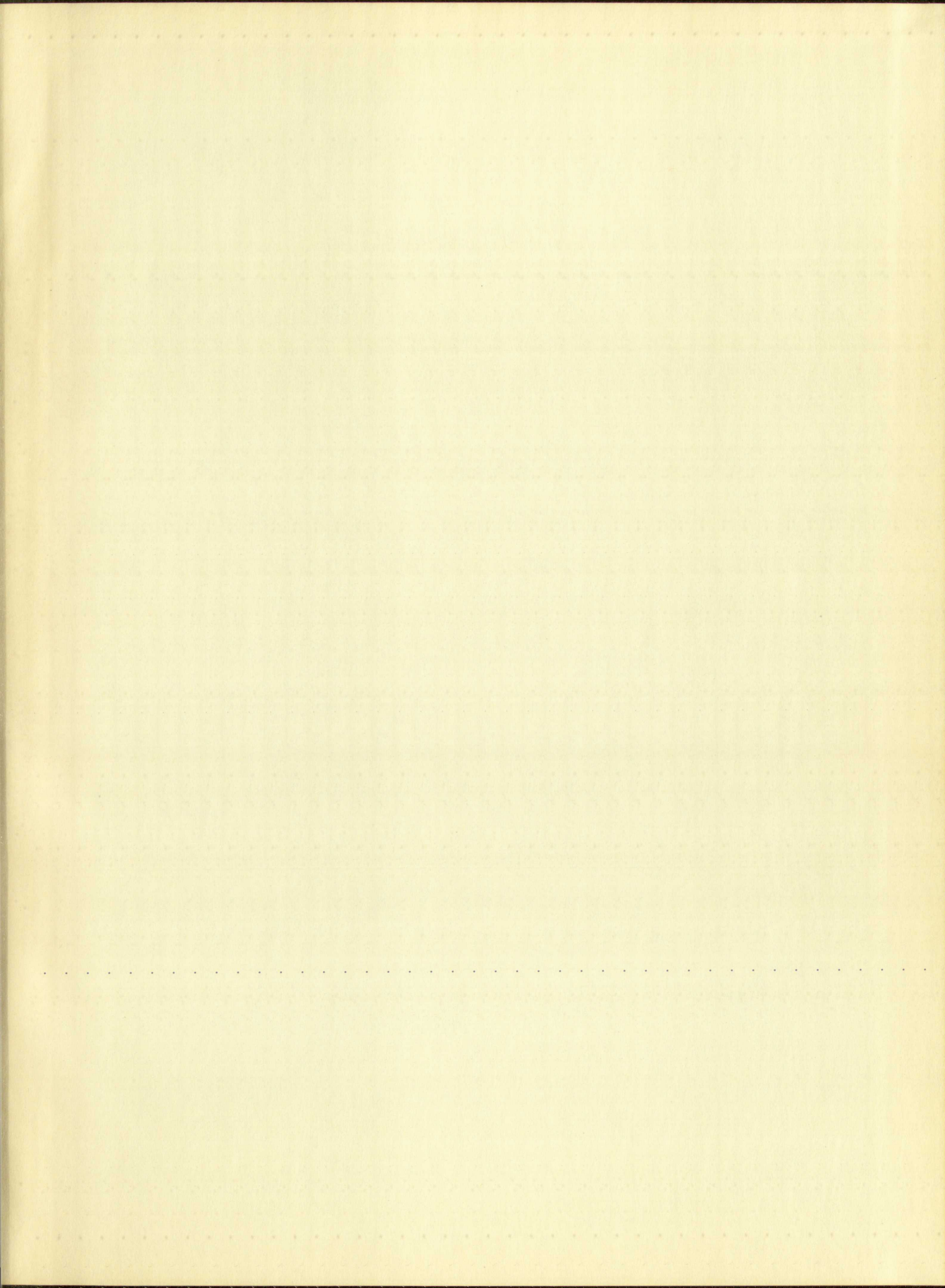
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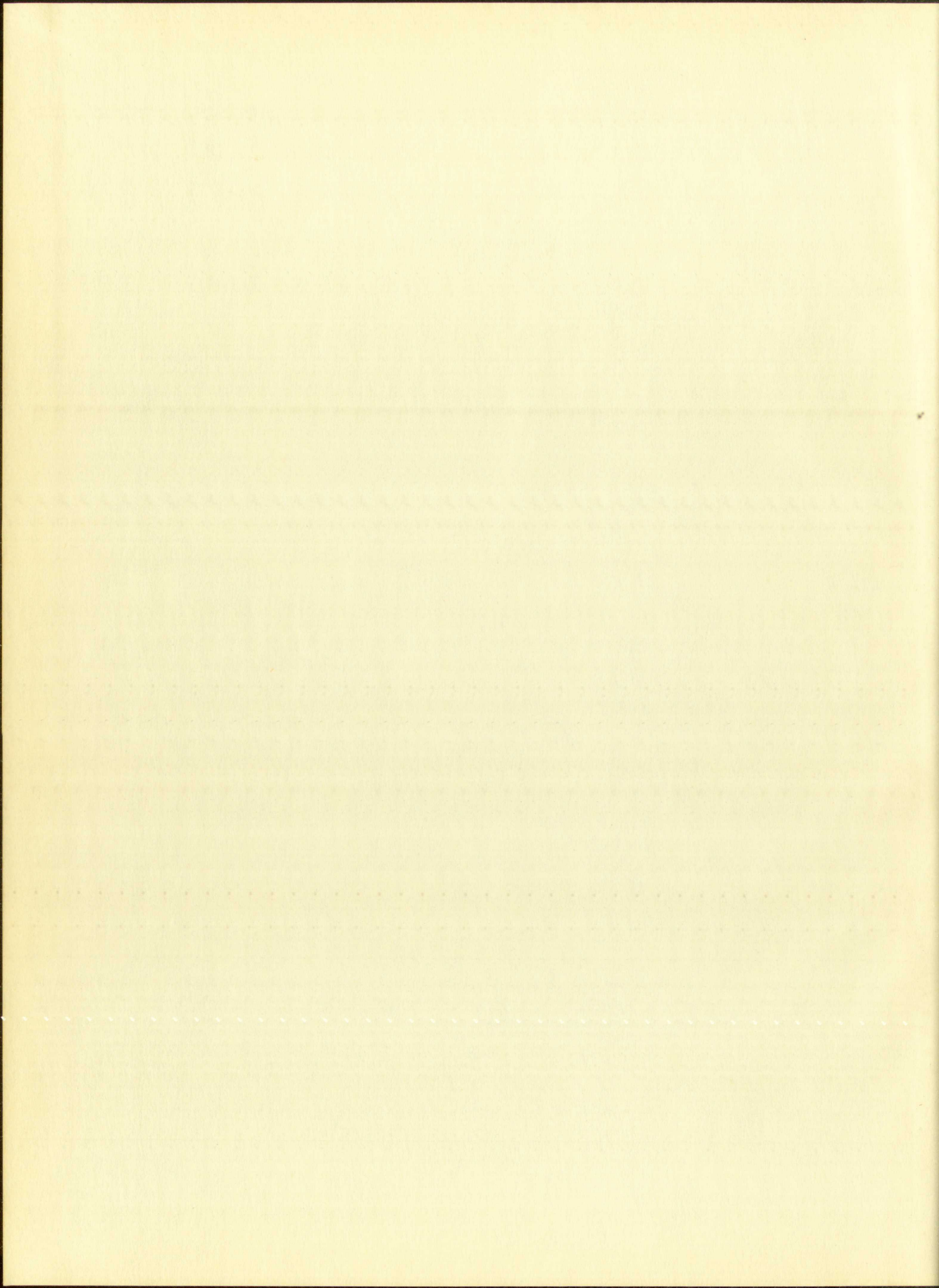
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A PHOTOMETRIC METHOD OF STUDYING  
THE CONTROL OF HANDEDNESS  
BY THE CEREBRAL CORTEX OF THE RAT

By

Donald K. Gucker

A Thesis

Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Arts in Psychology

The University of New Mexico

1959





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A Thesis  
Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Arts in Psychology

The University of Michigan

1962



This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

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University of New Mexico in partial fulfillment of the require-  
ments for the degree of

MASTER OF ARTS

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THESIS

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CHAIRMAN  
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"A science of the brain must point out  
the functions of its elements. A science  
of the relations of mind and brain must  
show how the elementary ingredients of the  
former correspond to the elementary  
functions of the latter."

William James, 1890



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W309  
1929  
2.92

"A science of the human mind is not  
the function of its elements. A science  
of the relations of mind and body must  
show how the elementary functions of the  
former correspond to the elementary  
functions of the latter."

Willard Van Dine, 1893

WILLARD V. DINE  
1893

24747



## TABLE OF CONTENTS

	Page
INTRODUCTION . . . . .	1
METHOD . . . . .	13
Apparatus . . . . .	15
PROCEDURE . . . . .	19
Tissue Preparation . . . . .	19
Densitometer Observations . . . . .	20
RESULTS . . . . .	22
DISCUSSION . . . . .	43
SUMMARY AND CONCLUSIONS . . . . .	50
REFERENCES . . . . .	51
APPENDIX . . . . .	54



TABLE OF CONTENTS

INTRODUCTION . . . . .	1
METHOD . . . . .	2
Apparatus . . . . .	3
PROCEDURE . . . . .	4
Tissue Preparation . . . . .	5
Densitometer Observations . . . . .	6
RESULTS . . . . .	7
DISCUSSION . . . . .	8
SUMMARY AND CONCLUSIONS . . . . .	9
REFERENCES . . . . .	10
APPENDIX . . . . .	11



LIST OF TABLES

	Page
Table I. Reaching records . . . . .	14
Table II. Predictive value of white matter data . . . . .	45
Table III. Predictive value of layer VI data . . . . .	46



Table I.	Reaching records
Table II.	Predictive values of the factor data
Table III.	Predictive values of the data

TABLES  
I, II, III



## LIST OF FIGURES

	Page
Fig. 1. Schematic of Densitometer . . . . .	17
Fig. 2. Lamination Curve . . . . .	48



LIST OF FIGURES

- Fig. 1. Schematic of Development . . . . .  
Fig. 2. Lamination Curve . . . . .

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## INTRODUCTION

In 1948 Hilgard wrote, "It is a blot upon our scientific ingenuity that after so many years of search we know as little as we do about the physiological accompaniment of learning." (Hilgard, 1948, p. 481) Today the situation remains unchanged; no one has been able to identify the change or modification occurring in neural tissue that permits learning and retention. The statement that structural modification occurs with learning is an assumption, but one that enjoys almost universal acceptance. Hilgard goes on to say that, "one of the most crying needs is for a crucial experiment identifying specifically a change occurring in neural tissues. . . as learning takes place." (Hilgard, 1948, p. 481) This thesis describes the first in a series of proposed experiments designed for identification of such a change.

Among the problems involved in such a program is the approach to be taken. The concept of structural change is many-sided and may involve change at a chemical level, a visible cellular level, or perhaps at some other unknown level. In addition to the nature of the change the problem of degrees of localization of learning has yielded various viewpoints that must be reckoned with.

Concerning the chemical approach, Rosenzweig, Krech,



# INTRODUCTION

In 1916 Hilgard wrote, "It is a little known fact that scientific ingenuity has often been hampered by the fact that we know as little as we do about the psychological mechanism of learning." (Hilgard, 1916, p. 101) Today the situation remains unchanged; no one has been able to justify the change or modification occurring in neural structures that permits learning and retention. The statement that structural modification occurs with learning is an assumption, but one that enjoys almost universal acceptance. Hilgard goes on to say that, "one of the most striking facts is for a crucial experiment identifying specific changes occurring in neural elements. . . or learning takes place." (Hilgard, 1948, p. 131) This leads to the first in a series of proposed experiments designed for identification of such a change.

Among the problems involved in such a program is the approach to be taken. The concept of structural change is many-sided and may involve changes at a cellular level, visible cellular level, or perhaps at some other intermediate level. In addition to the nature of the change the degree of degrees of localization of learning has been a matter of various viewpoints that must be reckoned with.

Concerning the cellular approach, Rosenzweig and



and Bennett (1958) ask the question, "What changes in the nervous system accompany learning? Though learning is generally considered to involve structural changes, and though the search for such changes has been the preoccupation of many experimenters and theorists, the complexities of the anatomy of the nervous system seem so far to have prevented the detection of such changes. A biochemical analysis which could integrate changes over thousands of neural units might provide an entering wedge to the solution of this problem. Further analysis might then focus more narrowly on the exact sites of change." One difficulty with this statement is the assumption that thousands of neural units are involved in a learning situation. At its present state of development the chemical approach is a gross one, and admittedly faces the problem of specialization as seen in localization of function.

Seeking a visible cellular change also presents formidable problems. Wyckoff and Young (1956) state, "The development of adequate methods for cutting thin tissue sections and improvements in techniques of fixation now make it possible to study the relations between the cells within the nervous system at the high resolutions of the electron microscope; the detail thus seen is bewildering in its complexity." In this dense forest of structure one wonders what type of change to seek. It is not im-



and Bennett (1973) saw the same thing. "The nervous system is generally considered to involve intricate changes, and though the search for such changes has been the preoccupation of many experimentalists and theoreticians, the details of the anatomy of the nervous system seem to have prevented the detection of such changes. The chemical analysis which would illustrate changes over thousands of neural units might provide an understanding of the solution of this problem. Further analysis might then focus more narrowly on the exact sites of change. One difficulty with this statement is the assumption that thousands of neural units are involved in a learning situation. At its present state of development, the chemical approach is a gross one, and naturally faces the problem of specialization as seen in the relation of function.

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possible for almost any of the numerous neural structures to participate in the modification, but theory and a scrap or so of evidence has narrowed "the field" down a bit. The structures that are encompassed in the concept of the synapse seem to appear in modification theory more often than other neural structures, and this seems to be due to the fact that it is easier to envisage change at multiple neural junctions than at other places in the system. Hebb (1949) postulates a thickening of fibers and/or enlargement of synaptic knobs as two possibilities. Eccles (1953) has illustrated at the level of the cord that the physiological properties of the synapse are modifiable depending on use and disuse. Specifically he modified the transmissive abilities of the synapses by controlling the amount of use they were subjected to and thus lent support to the theory of change at the synaptic junction. Eccles explains his evidence in terms of postulates concerning change in size of boutons with use and disuse (1953). Learning theory has utilized synaptic structures with little regard for the fact that our information concerning these structures came from investigations of . . . . . spinal neurons. Only recently has evidence been presented concerning the presence of similar structures in the cerebral cortex.

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Smeythies, Gibson, and Parkis published a paper on "The Distribution and Morphology of Bodily Structures"



in the Human Cerebrum." (1957) Their study seems to establish the presence of boutons in the cortex, but their conclusions concerning their distribution seem questionable, considering that a silver stain was used. The important point here is that the boutons do seem to exist in the cortex; this at least lends the air of feasibility to theories which utilize boutons as a mechanism involved in learning.

Recently Palay (1956a) demonstrated electron micrographs of boutons present in the medulla. Under the tremendous magnification of the electron microscope a complex typical internal structure is revealed. This structure is characterized by the presence of mitochondria and small vesicles. In a publication concerning synapses in the central nervous system Palay states: "In other synaptic fields, such as the molecular layer of the cerebellar cortex and in the cerebral cortex, formations with this essential structure also appear. In these regions, the axon terminals are small and contain only a few mitochondria and a cluster of vesicles located near a thickened limiting membrane in apposition to a typical dendritic process." (Palay, 1956b, p. 198) His study utilized the brain of the albino rat.

The staining of boutons is difficult, patchy, and unreliable; consequently any attempt to use their number or their size as an index of change would face grave



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The staining of boutons is difficult, but not  
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difficulties. Eccles, in his discussion of plasticity, perhaps points to a method of avoiding the "bouton problem." He states,

"The most probable postulate for post-tetanic potentiation is that the presynaptic impulse becomes a more effective synaptic excitor (or inhibitor), because repetitive stimulation temporarily alters the spatial relationship of the synaptic knobs to the post-synaptic membrane; for example, the knobs may become larger and/or in closer apposition thereto. The volume changes observed in repetitively stimulated giant axons (Hill, 1950) may be expected to occur also in the non-medullated presynaptic fibres during and after repetitive stimulation, for the uptake of water in considerable part at least is determined by the flux of ions that is associated with the nerve impulse. The relatively large surface to volume ratio associated with the very small diameter of these fibres (probably no more than 0.2 to 0.3  $\mu$ ) would lead one to expect a relatively greater swelling and possibly a more rapid time-course of the swelling and recovery therefrom. Furthermore, given uniform elastic properties of the membrane, the synaptic knob, having a larger radius of curvature than its attached fibre, would be expected to swell more readily than this fibre in response to an increase in turgor of the system; i.e., there would be a flow of axoplasm from the swollen fibre to increase



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further the swelling of the knob.

If it is assumed that the uptake of water per square centimeter of surface is the same as in the giant fibre (where it is rather more than  $10^{-5}$  cc. per sq. cm. of membrane for 10,000 impulses), then there is a surprisingly large swelling of the fine presynaptic fibre and knob. A 0.3 diameter fibre would more than double its volume, swelling to  $0.46\mu$  diameter, and a  $1.0\mu$  diameter (spherical) knob would swell to  $1.2\mu$ ." (Eccles, 1953, pp. 198-199)

Consequently we might conclude that if we cannot examine boutons because of difficulties in staining, then perhaps changes in turgor may be reflected in more stainable structures such as fibers and cell bodies. If internal pressure is increased to the point of swelling the fiber, then it is not unlikely that small concomitant changes in the volume of the cell body may occur. Even if these changes are only in the order of a tenth of a micron, but occur in a number of cells, they might be detected by a sensitive instrument such as Hill (1950) used in measuring small volume changes in squid axons. It is this general line of reasoning that this experiment follows. Before entering the particular nature of this investigation, however, one other point must be mentioned. The physiological psychologist must decide before attempting to investigate learning histologically, where



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in the nervous system this learning is located. Sperry (1958, p. 401) summed up one point of view when he wrote, "Cortical lesions produced initially in the expectation that whole blocks of memory and categories of experience and behavior would be wiped out proved in many cases to have so slight an effect on functional organization and memory as to tax the ingenuity of the investigator to detect any behavioral defects. The question of the locus and nature of the memory trace or engram became recognized, through the work of Lashley, in particular, as one of the most baffling of all neurological problems. Almost any engram scheme that might be expressed within the framework of the traditional approach to cerebral integration, i.e., in terms of specific fiber connection, seemed to be ruled out."

Lashley (1950, p. 477) treated the subject of localization most directly when he wrote, "all the cells of the brain are constantly active and are participating, by a sort of algebraic summation, in every activity. There are no special cells reserved for special memories." The alternative school of thought, that is there are specific loci of learning, has recently been defended by Hebb in his book, The Organization of Behavior (1949). Hebb states, "The assumption we must accept is that the memory trace, the basis of learning, is in some way structural and static." (p. 12) There are two primary obstacles to



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the acceptance of this assumption; the first being the work of Lashley and the second is the work of the Gestalt psychologists. Kohler has developed a theory of electrical fields in the brain to account for perception and learning. Gestalt investigators emphasize pattern rather than locus, and it is only fair to say that they have considerable experimental data for support. Hebb spends the major portion of his book trying to show that their conclusions are not justified by their evidence. Our primary concern here is with the work of Lashley, because it is much closer to the subject at hand than the work of the Gestaltists. Hebb (1949, p. 13), when speaking of Lashley's work, points out, "that the removal of blocks of the rat's cerebral cortex does not affect habits selectively. If one habit is affected, others are also. From this Lashley has concluded that memory traces are not localized in the cerebral cortex, but himself has pointed out another possible interpretation. His evidence is consistent with the idea that the trace is structural, but diffuse, involving, that is, a large number of cells widely spaced in the cortex, physiologically, but not anatomically unified." "This is not, consequently, crucial evidence for or against the notion of structural traces in the cortex." If specific loci are not important, then how can repeated sensations that reinforce one another result in the relatively permanent effect we call learning? If we



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accept a hypothesis concerning reverberatory activity among neural chains with no specific loci, it becomes difficult to imagine how these activities would not interfere with each other and how they could survive violent cortical storms such as grand mal epilepsy or cerebral concussion. As Hebb states, "a pattern of activity not dependent on structural changes for its permanence--this seems in the highest degree unlikely." (Hebb, 1949, p. 13)

We cannot strictly prove Lashley's statement concerning "no special cells" to be incorrect. However, I have cited some evidence and thought that does not support his conclusions.

Practically speaking, it is easier to search in some specific place than any place in general by some specific means. Any general approach must contend with the microscopic size of the cortical elements presumably involved in learning, and also with the concept that different instances of learning may involve different types of change. Analytical devices available for such general approaches do not take these questions into account, and consequently on a practical level one finds advantages in searching for a structural change in a specific locus.

In the gross pursuit of the structural trace, one encounters the problem of cortex versus sub-cortical structures, and in this delineation we find some of Lashley's work useful. It has been held that memory traces



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are first formed in cortex and then transferred to sub-cortex. This notion results from the greater instability of recently-formed habits than of old habits, and from the assumption that consciousness is a cortical function. Old habits become automatic and are performed without conscious control; consequently they are not located in the cortex. This reasoning has been discredited by Lashley's work on the discrimination of brightness in the rat. Removal of the striate cortex abolishes the brightness habit completely, but with new practice the animals are able to relearn the habit. Tremendous overlearning in a group of animals produced no change in the results. "The long, overtraining did not eliminate the participation of the cortex. . . . After animals, lacking the visual cortex, have learned a brightness habit, any other part of the cortex may be destroyed without disturbance of the habit. Apparently no other part of the cortex takes over the learning function." (Lashley, 1950, p. 466) Lashley showed that the particular sub-cortical structure involved does not participate in the learning as long as the necessary cortex is intact. He sums up his experimental work in this area, "If the cerebral cortex is intact, the associative connexions of simple conditioned reflexes are not formed in the sub-cortical structures of the brain." (Lashley, 1950, p. 467) There have been numerous studies concerning the possibility of condi-



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numerous studies concerning the possibility of habit-



tioning occurring at lower levels in the central nervous system (Morgan & Stellar, 1950, pp. 439-451), but they are unimportant here because intact cortex is prepotent; consequently, it is here that our interest lies.

Specifically, our question boils down to this: where are the cortical loci of learning and what specific modifications are concomitant with this learning?

Handedness in the rat has been selected as a behavioral function subject to modification by practice (Peterson, 1934). In searching for a structural correlate handedness yields several advantages. First, it is easily determined by a food reaching situation (Peterson, 1934). Second, it is modifiable by practice and therefore subject to a form of learning. Third, its cortical representation has been highly localized in the rat (Peterson & Gucker); and lastly, it has a unique double cortical representation (when the non-preferred hand is included) with an area in each hemisphere. One of these areas controls the preferred limb and the other controls the non-preferred limb. The simultaneous existence of these two areas, one subject to practice and the other not, offers a basis of comparison unique in the search for a structural correlate. The objective of this investigation is to compare histologically, the preferred cortical area with its homologue and seek a structural difference in animals with known hand preference.







Considering our lack of knowledge concerning the functional significance of neuro-anatomical elements, a rather unique method has been devised for histological observation and deserves brief mention at this point. As stated previously, one feasible change is of size due to increased turgor pressure in a closed system.

Hill (1950) detected and measured small changes in the size of the giant axon of the squid by optical means. He measured the quantity of light entering a photocell after passing through an optical arrangement in which the axon was inserted. This investigation uses the same principal but apparatus that is quite different from Hill's. This method does away with the need for subjective evaluation of structural elements and thereby meets the objections voiced by Lashley and Clark (1946) concerning such methods of observation. Using this apparatus in conjunction with stains selective for a particular cortical element allows inquiry into the parts played by these elements in the role of learning.

Consequently, this study is designed to compare the amount of light impedance occurring in the cortical areas controlling the preferred and the non-preferred limb in the rat, in an attempt to relate optical density of structure to preferential handedness.



Considering our lack of knowledge concerning the functional significance of these anatomical structures, rather unique method has been devised for their observation and observation and observation at this point. As stated previously, one possible method is to observe increased target pressure in a closed system. Hill (1950) detected and measured small changes in the size of the plant axon of the plant in relation to the quantity of light entering the plant. He measured the quantity of light entering the plant after passing through an optical arrangement in which the axon was inserted. This investigation was the first optical but suggests that the plant is a living system. This method does away with the need for a sensitive reaction of structural elements and thereby avoids the objections voiced by Lashley and Hill (1950) concerning such methods of observation. Within this experimental conjunction with a single selective for a particular element allows inquiry into the exact structure of elements in the role of learning. Consequently, this study is designed to determine the amount of light impinging upon the plant and the controlling the pressure and the response of the plant to the test, in an attempt to determine the role of structure to potential functional changes.



## METHOD

Subjects

Ten male hooded rats were selected after testing in the Peterson food-reaching situation. Any rat that did not meet the requirements of a single hander was removed at this point. Single-handed rats are defined for purposes of this study as those taking not more than two percent of total reaches with the non-preferred hand. A total of thirty-two rats were tested in order to find ten such single-handed animals. Table I presents the reaching records of these animals. It will be seen that there were five right-handed and five left-handed rats.



## Subjects

Ten male hooded rats were selected for study in the Peterson foot-reaching experiment. Any rat that did not meet the requirements of a single-handed rat was removed at this point. Single-handed rats were selected for the purpose of this study as those tending not more than 50 percent of total reaches with the nonpreferred hand. A total of thirty-two rats were tested in order to obtain ten such single-handed animals. Table I gives the reaching records of these animals. It will be seen that there were five right-handed and five left-handed rats.



Table I -- Reaching records

<u>Rat No.</u>	<u>Left reaches</u>	<u>Right reaches</u>	<u>Handedness</u>
1	0	450	R
2	0	450	R
3	0	450	R
4	2	448	R
5	4	446	R
6	450	0	L
7	450	0	L
8	450	0	L
9	447	3	L
10	442	8	L



Table 1 -- Bushyland records

<u>Rat No.</u>	<u>Left trachea</u>	<u>Right trachea</u>	<u>Examination</u>
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	450	0	0
7	450	0	0
8	450	0	0
9	450	0	0
10	442	0	0

WILSON COLONY  
 1925  
 WILSON



### Apparatus

The primary piece of apparatus is an ultra-sensitive and stable densitometer with its associated equipment. The densitometer utilized a vacuum phototube as a light receptor. This phototube was placed in a 35 mm. camera body mounted on the third ocular of an A/O trinocular microscope. A slide placed on the mechanical stage of the scope was subjected to a light beam of variable diameter coming up from the illuminator and passing through the sub-stage condenser. The Sleigh and Khune microscope illuminator was voltage-regulated by a Sola #30886 regulator operated at full load. Instrument stability seemed adequate as evidenced by no observable fluctuations in the indicator circuit during operation. After passing through the tissue the light beam enters the 10 x objective of the scope and passes through its usual optical system. The photocell receives the beam after it enters the camera objective. The cathode is placed slightly above the focal plane of the camera so that the image will be slightly out of focus and thus negate any local variation in the image or cathode surface. The phototube is mounted in a metal box that is attached to the camera, and it is grounded to the shielded cable that connects the phototube to the amplifier.

A densitometer is an instrument which measures the amount of light transmitted by a substance, and the photo-



The primary piece of apparatus is an electron microscope and stable densitometer with its associated equipment. The densitometer utilized a vacuum photodiode as a light receptor. This photodiode was placed in a light-tight body mounted on the third column of an electron microscope. A slide placed on the specimen stage of the scope was subjected to a light beam of electron beam after coming up from the illumination system through the sub-stage condenser. The slide and beam were scope illuminator was voltage-regulated at a 100-1500 regulator operated at full load. Instrumentation seemed adequate as evidenced by no significant variations in the indicator circuit during operation. Light passing through the tissue the electron beam and the 10 x objective of the scope and camera through the optical system. The photodiode received the beam as it enters the camera objective. The detector is placed slightly above the focal plane of the camera so that the image will be slightly out of focus and magnified. Local variation in the image or density variations in the photodiode is mounted to a metal box and is connected to the camera, and it is grounded to the electron microscope. connects the photodiode to the amplifier.

A densitometer is an instrument which measures the amount of light transmitted by a specimen and the



cell with amplifier stages described here constitute such an instrument. The d.c. amplifier circuit is one published by RCA (Mark, 1956) and designed specifically for use as an ultra-sensitive wide-range unit. A schematic is presented in Figure 1. The photocell constitutes one arm of a bridge circuit that uses  $R_1$  as a balance. The voltage reference and regulator circuit has been modified to include a 6627 tube and thus provide a closer degree of reference than the OC3/VR105 originally specified by RCA. The B+ voltages are supplied by a Tektronix 160A power supply, thus providing a B+ of 225 volts when line voltage varies between 105-125 volts. The filament 6.3 VAC also came from the power supply but was not regulated. Since a regulated B+ was used in conjunction with the reference circuit in the amplifier, excellent drift-free stability was obtained. Several prototype amplifiers were constructed before a suitable instrument was produced. It proved necessary to use high quality precision 1 percent resistors throughout in order to reduce all possible sources of noise. It was found necessary to shield the entire unit in a metal case; otherwise a person near the instrument caused deflection of the galvanometer.



cell with amplifier and read-out device connected to an instrument. The amplifier is of the type described by RCA (Mark, 1955) and designed specifically for use as an ultra-sensitive wide-range amplifier. The circuit is shown in Figure 1. The power supply is a bridge circuit that uses a transformer, a bridge circuit, and a regulator circuit. The bridge circuit includes a 600V tube and other electronic components. The reference than the 600V tube is a 600V tube. The B+ voltages are regulated by a transformer and a supply, thus providing a B+ of 125 volts when the voltage varies between 105-135 volts. The power supply comes from the power supply and is regulated. A regulated B+ was used in the amplifier circuit in the amplifier, excellent results were obtained. Several prototype amplifiers were constructed before a suitable design was obtained. It proved necessary to use high quality components, resistors throughout in order to minimize the sources of noise. It was found necessary to mount the entire unit in a metal case; otherwise a ground loop instrument caused deflection of the galvanometer.



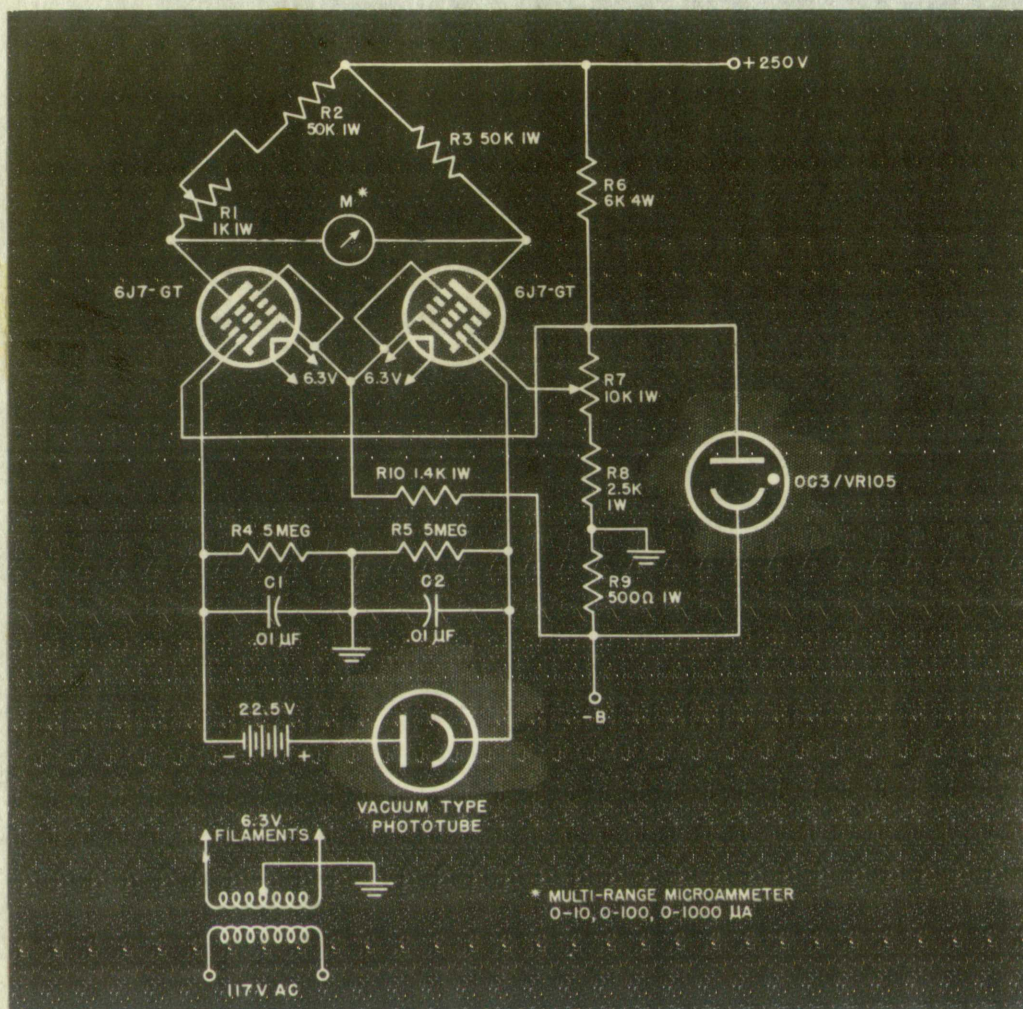


Figure 1 -- Schematic of Densitometer

The difference in d.c. potential on the plates of the 6J7's is applied to the floating inputs of a Kintell model 204 Electronic Galvanometer. This instrument provides a d.c. chopper amplifier stage with an Aryington shunt at the input. This is a completely transistorized unit that exhibits high stability and sensitivity. The shunt was set so that a difference of 100  $\mu$ a applied to the input



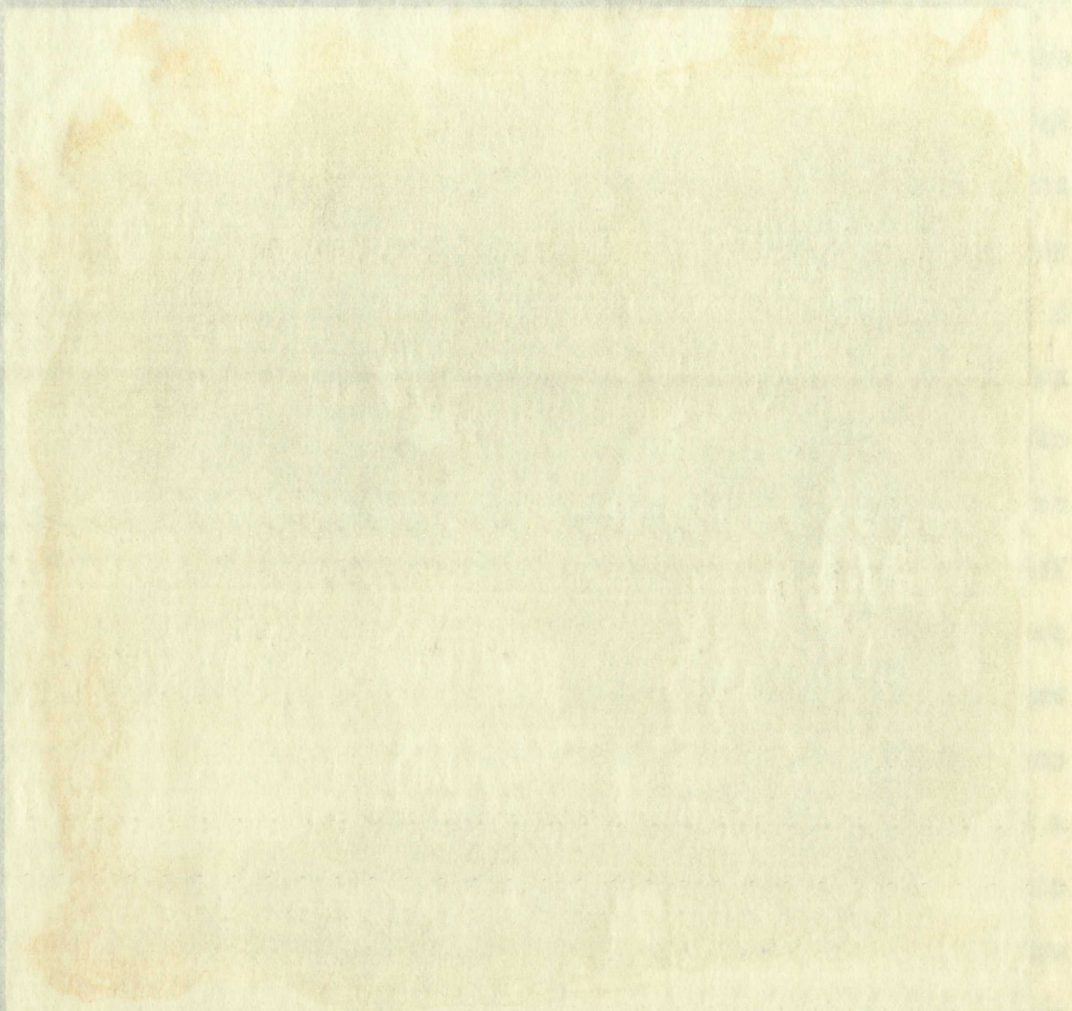


Figure 1 -- Schematic of the system

The difference in the two signals is the error signal. This error signal is applied to the input of the 204 Electronic Calculator. This calculator provides a d.c. chopped amplifier output which is fed back to the input. This is a complete feedback system which exhibits high stability and accuracy. The error signal is set so that a difference of 100% is maintained between the two signals.



yielded a full scale deflection. Balance was obtained by using  $R_1$  as a coarse balance and adding a fine control in series. A rough idea of the extreme sensitivity provided by the amplifier is found in the statement, "The sensitivity of this circuit will provide a usable meter deflection for phototube currents as low as  $.0001 \mu \text{ a.}$ " (Mark, 1956, p. 78) The circuit provides an essentially linear relation between meter current and illumination.

The instrument reflects the amount of light transmitted, primarily by the stained structures in the tissue, as a quantitative reading on the galvanometer, thus providing a measure of the density of stained elements.



yielded a full scale deflection. The scale was calibrated using R<sub>1</sub> as a coarse balance and a standard resistor series. A rough idea of the accuracy of the scale by the amplifier is given in the statement. The accuracy of this circuit will be less than a standard meter used for photometric measurements (see, for example, p. 78). The circuit provides an accuracy of about 1% in the relation between meter current and deflection. The instrument calibrates the current of the standard resistor, primarily by the standard resistance in the circuit as a qualitative reading on the galvanometer. The primary reading a measure of the density of the stained specimen.



## PROCEDURE

Tissue Preparation

After taking the last 50 reaches out of a total of 450 in the handedness test situation, the animal was sacrificed under ether, the brain removed and placed into 10% formol. After at least 24 hours in the fixative, serial 40 sections were cut by the freezing technique and mounted with Mayers Albumen, four to a slide. The cortical area sectioned was determined by previous localization studies (Rivoire, 1957; Peterson & Gucker, in press) and included that between the anterior pole of white matter and the dorsal convexity of the caudate nucleus. Usually 16 to 24 serial sections 40  $\mu$  thick encompassed this area. The hemispheres of a single brain were sectioned separately, due to a lack of bilateral symmetry in the location of the white matter and caudate nucleus, with the left always being sectioned first. Small variations in section thickness are allowed to vary at random due to the difficulty in control in the freezing technique. Since five left-handers and five right-handers are used, any influence due to sectioning of the left hemisphere first is controlled. The freezing technique is preferred in this investigation because it does not involve the shrinkage and difficulties involved in the celloidin and paraffin methods. After mounting on slides



## PROCEDURE

### Tissue Preparation

After fasting the last 24 hours of a rabbit was killed by bleeding from the abdominal aorta. The brain was removed and placed in 10% formalin. After at least 24 hours in the fixative, serial 10  $\mu$  sections were cut on a freezing microtome and mounted with Mayer's Albumen, fixed to a slide, and cortical area sectioned was determined by cytochrome oxidase reaction (Nivolar, 1957; Peterson & Gundersen, 1969) and included that between the anterior commissure and the dorsal boundary of the white matter. Usually 10 to 20 serial sections 10  $\mu$  thick were sectioned separately, due to a lack of symmetry in the location of the white matter and nucleus, with the left always being sectioned first. Small variations in section thickness are allowed to vary at random due to the difficulty in controlling the thickness. Since five left-hand and five right-hand sections are used, any influence due to sectioning of the left hemisphere first is cancelled. The use of a double-blind technique is preferred in this evaluation because it does not involve the author and a limited section in the cerebellum and parietal regions. After sectioning on slides



both hemispheres are placed in the oven at 35-40°C. for at least 24 hours. The two hemispheres of each brain are treated as much alike as possible. Different brains may be treated differently, but this produces no loss of control since the comparisons are to be made between the two hemispheres of a single brain and not between brains.

After drying in the oven, both hemispheres are placed back to back in an alternate fashion in a ten-slot staining dish. The purpose of this arrangement is to subject both hemispheres to the same staining conditions. The sections are then stained by the Kluver Barraer technique for fibers and cell bodies. A detailed description of this method is included in the appendix. After staining, the slides are mounted with balsam and number two cover glasses.

#### Densitometer Observations

The observers, GP and KE, were unaware of the handedness of the rats involved in the study except for knowing that five were left-handers and five right-handers. The identification marks on the slides gave no indication of handedness. Each observer made two observations on each slide, one in the white matter and the other in layer VI of the cortex. The densest spot in the white matter was searched for and recorded because several preliminary cases suggested a relation between density of fibers and



both hemispheres are placed in the oven at 50-60°C. for at least 24 hours. The two hemispheres of each brain are treated as much alike as possible. Different methods may be treated differently, but this procedure is best to follow since the comparisons are to be made between the hemispheres of a single brain and not between brains. After drying in the oven, both hemispheres are placed back to back in an aluminum basket in a staining dish. The purpose of this staining dish is to subject both hemispheres to the same staining conditions. The sections are then stained by the silver method, which is used for fibers and cell bodies. A detailed description of this method is included in the appendix. After staining, the slides are mounted with balsam and then covered with cover glasses.

### Behavioral Observations

The observers, GP and ME, were unaware of the handedness of the rats involved in the study except for the fact that five were left-handers and five were right-handers. Identification marks on the slides were made by the observers. Each observer made two observations on each slide, one in the white matter and the other in the gray matter of the cortex. The denser spot in the white matter was searched for and recorded because it was the most common case suggested a relation between handedness and the location of the denser spot.



handedness. With the Kluver stain a band of lightly stained fibers occurs just above the white matter in layer VI. One observer, KE, searched for the lightest area in this band and recorded that density; and GP selected a random sample from this area, thus giving two types of readings in layer VI. Since fibers stain lightly in this band, the comparative influence of cell bodies is probably rather strong. An arbitrary slide was selected, usually in the left hemisphere, and the bridge balanced to a zero setting on the galvanometer. The slides in that hemisphere and the other derive their absolute readings from this relative zero. The zero point is different for not only each rat, but also between white matter and layer VI readings in the same animal. The extreme sensitivity of the instrumentation required this reset procedure. The diameter of the light beam used for readings in the white matter was approximately  $240\mu$ , and that used for layer VI was approximately  $560\mu$ . The diameter of the beam was set before each set of readings and not changed until each particular set was completed.

.....



handiness. With the River station, a number of layers  
stained fibers occurs just above the surface in layer  
VI. One observer, H.E., searched for the stained fibers  
this band and recorded that certain of the stained  
random sample from this area, showing the fibers  
readings in layer VI. Since the influence of each  
band, the comparative influence of each band is  
rather strong. An arbitrary side was selected, usually  
in the left hemisphere, and the right hemisphere was  
reading on the galvanometer. The side in the left  
sphere and the other derive their values from  
this relative zero. The zero point is different for  
only each rat, but also between white water and layer VI  
readings in the same animal. The extreme sensitivity of  
the instrumentation required this procedure. The  
diameter of the light beam used for readings in the water  
matter was approximately 500  $\mu$ , and that used for layer  
VI was approximately 500  $\mu$ . The diameter of the beam  
was set before each set of readings and was changed after  
each particular set was completed.



## RESULTS

Graphs are presented, ten for the white matter and ten for layer VI, that compare the density of the preferred hemisphere with that of the non-preferred hemisphere. The heavy line represents the density of the various sections of tissue, starting at the left of the baseline with the beginning of the white matter and moving to the head of the caudate nucleus at the right, of the preferred hemisphere the thin line represents the same density relations for the non-preferred hemisphere.

The graphic results of KE were so similar to GP's that computation and graphic material presented here are those of GP. One reason for the selection of his data is that the layer VI material resulted from a more or less random sampling of the cortical area, while KE selected the lightest spot available in the area.



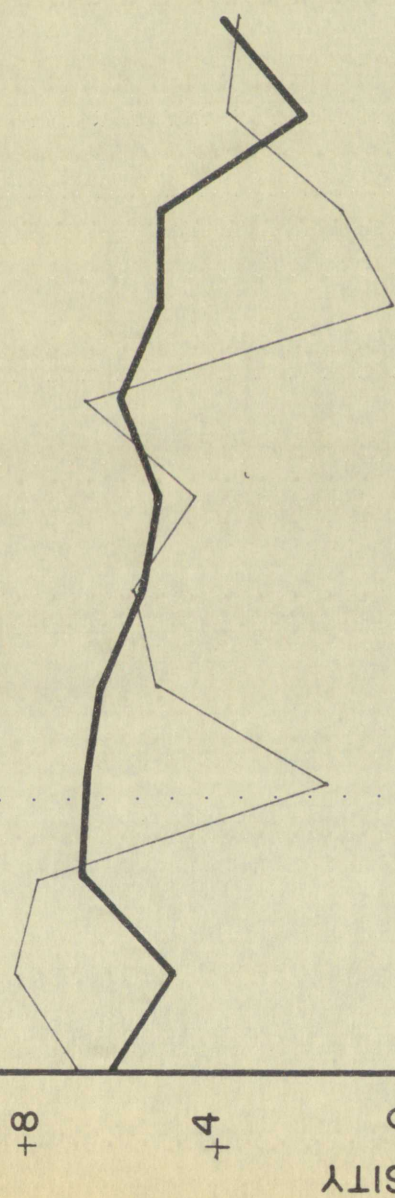
## RESULTS

Graphs are presented, first for the white matter and then for layer VI, that compare the results of the two hemispheres with that of the non-preferred hemisphere. The heavy line represents the density of the white matter of tissue, starting at the left of the graph and ending at the beginning of the white matter and ending at the right of the graph. The thin line represents the density of the white matter for the non-preferred hemisphere.

The graphic results of the white matter and layer VI that comparison and graphic results of the white matter and layer VI. One reason for the selection of the white matter is that the layer VI material resulted from a more or less random sampling of the cortical area while the white matter selected the lightest spot available in the area.



I WM









I VI

+8

+4

0

-4

-8

DENSITY

1

2

3

4

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6

7

8

9

10

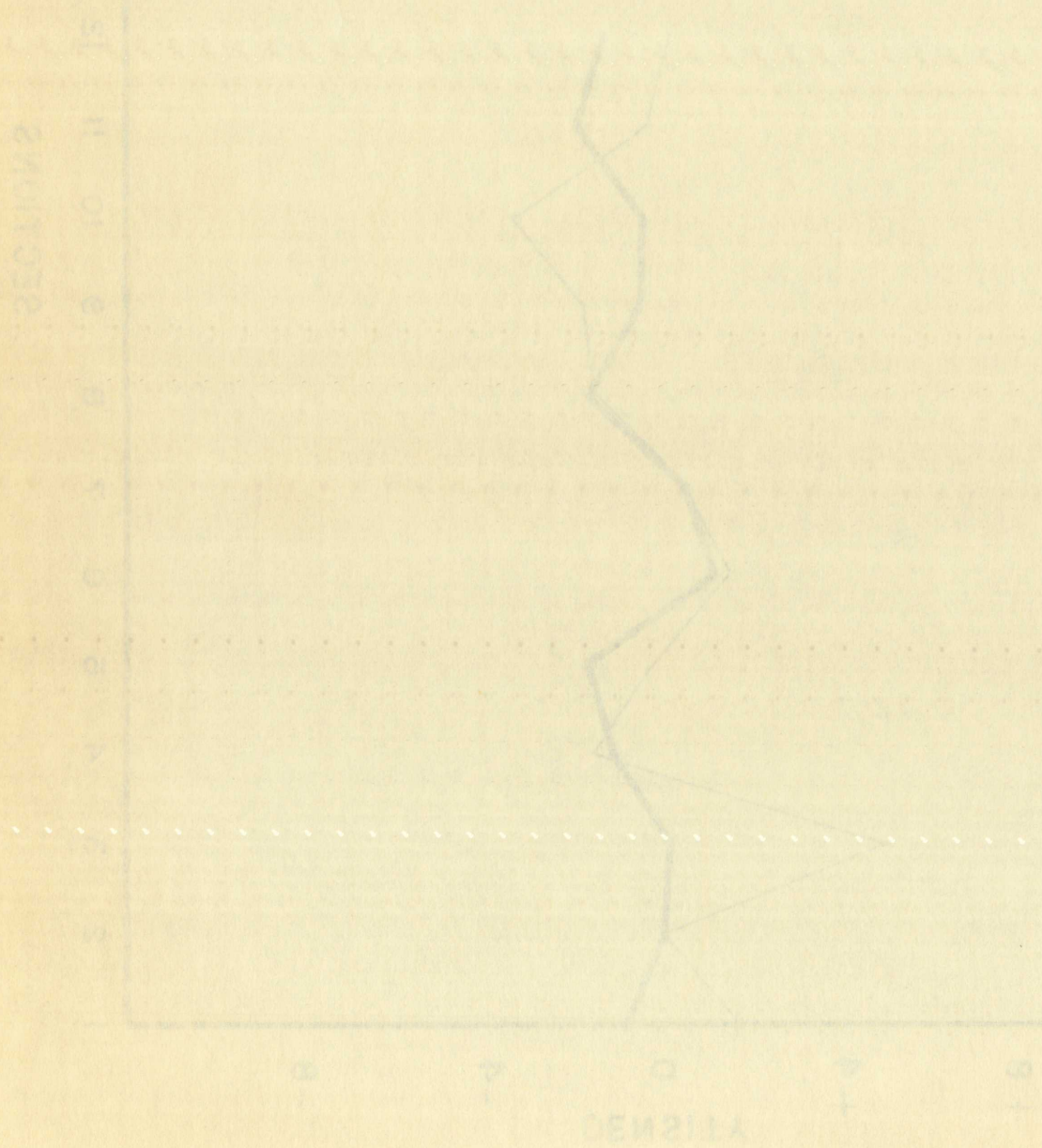
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12

SECTIONS



12





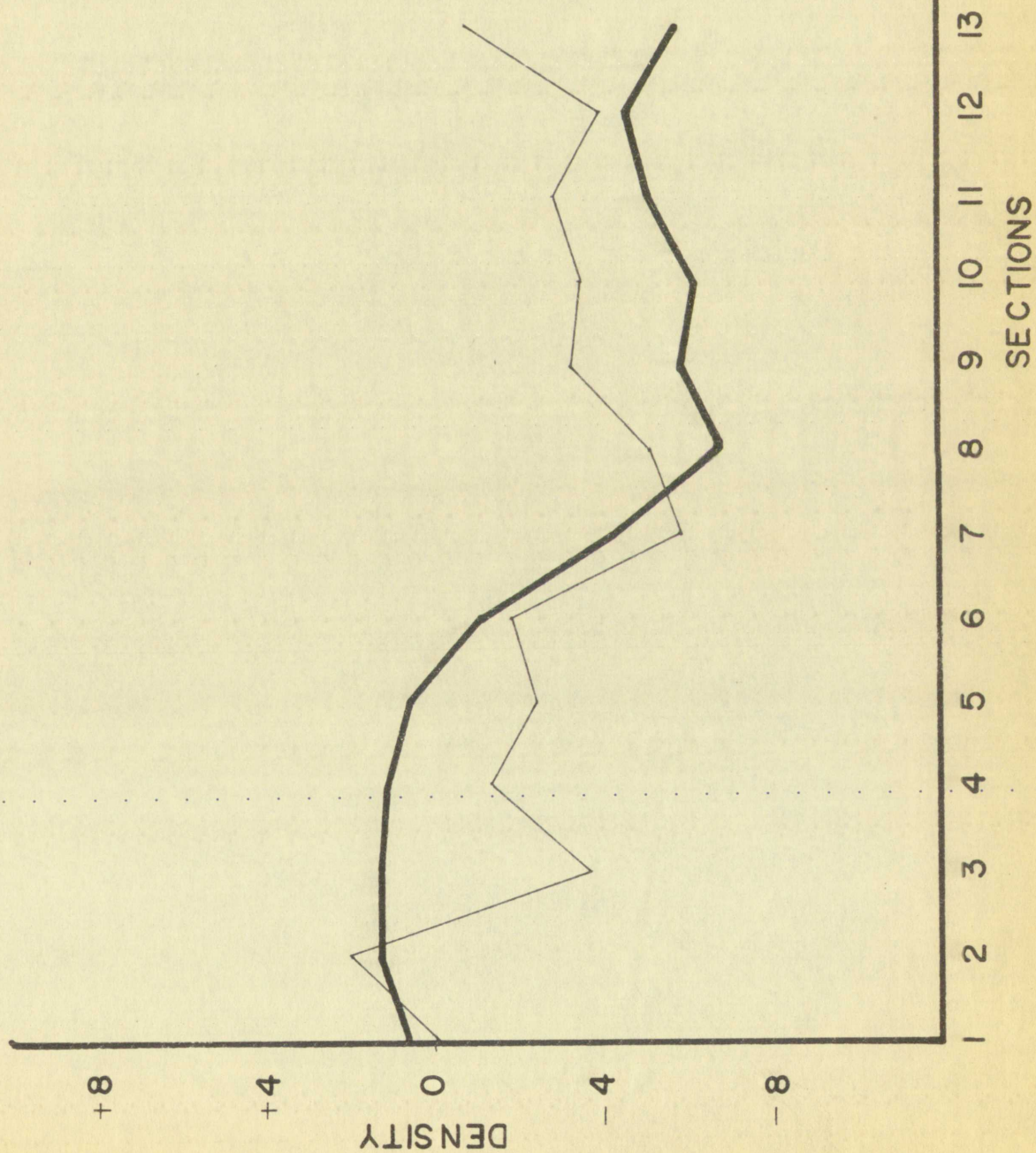
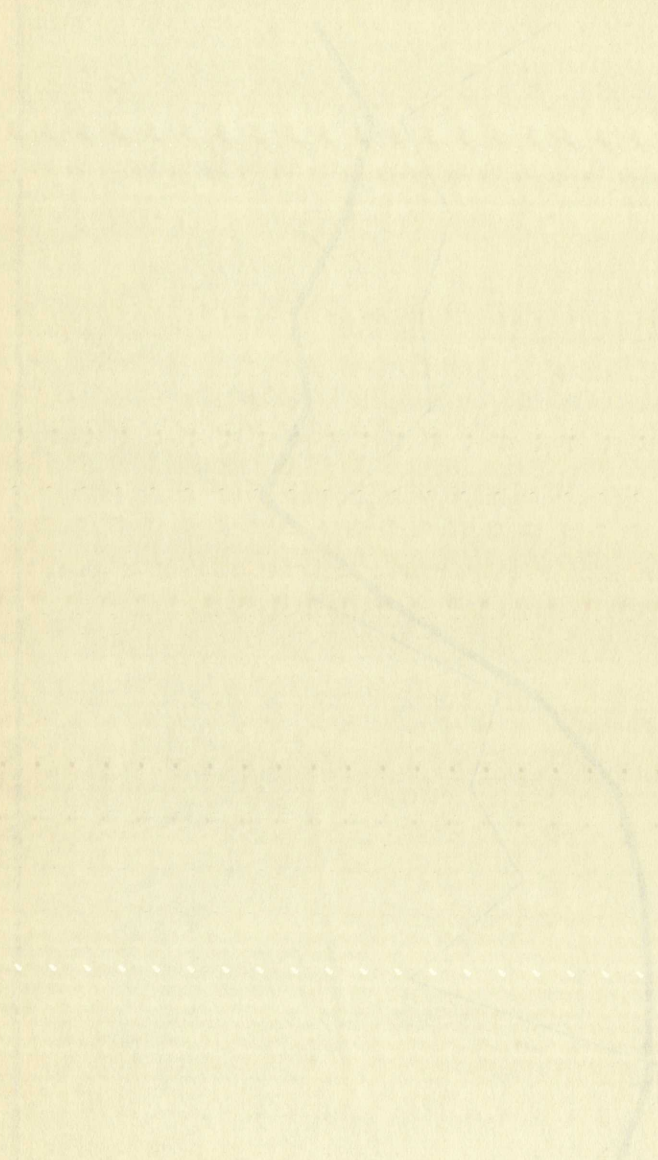




Figure 5

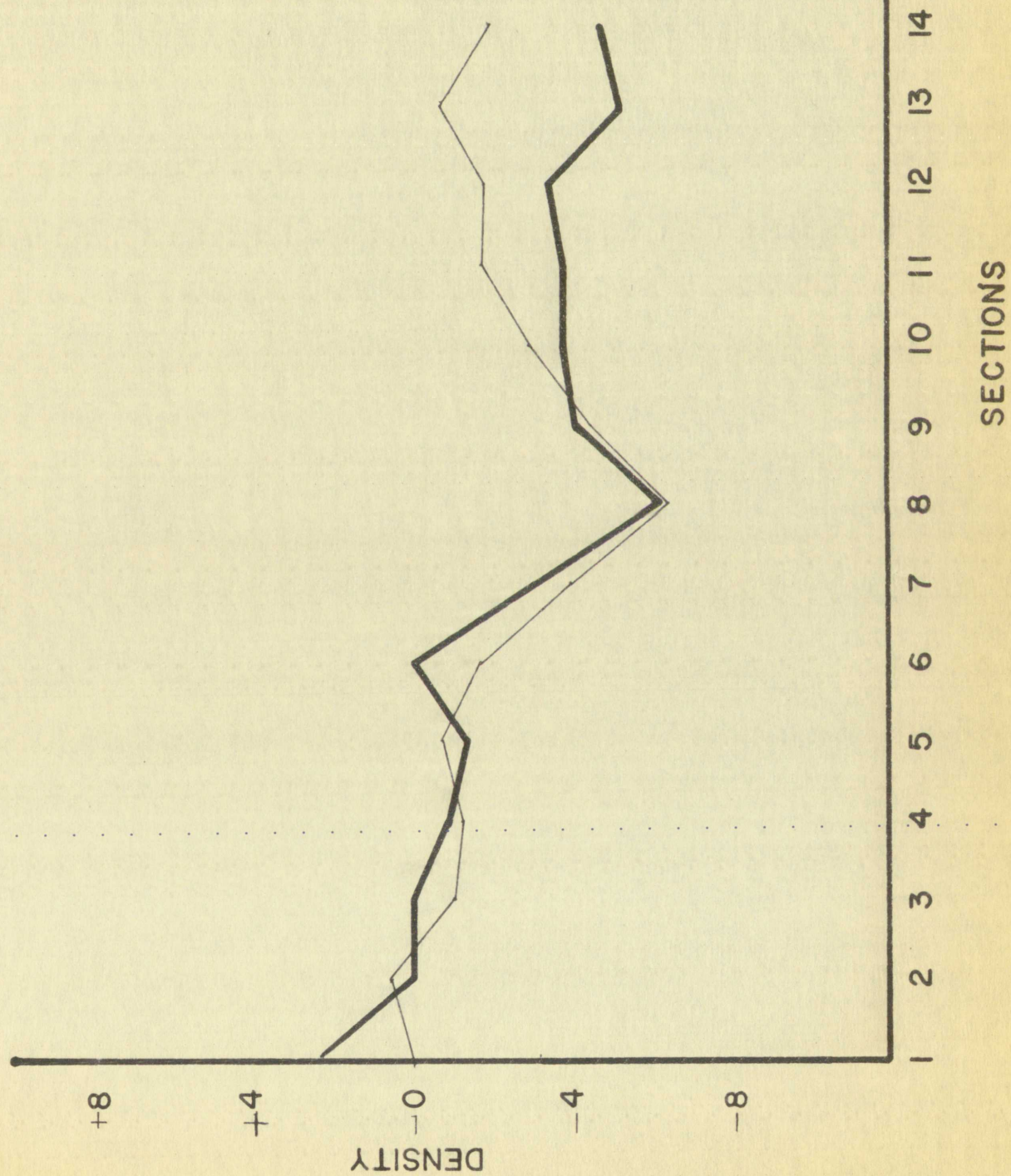


0 1 2 3 4 5

Figure 5

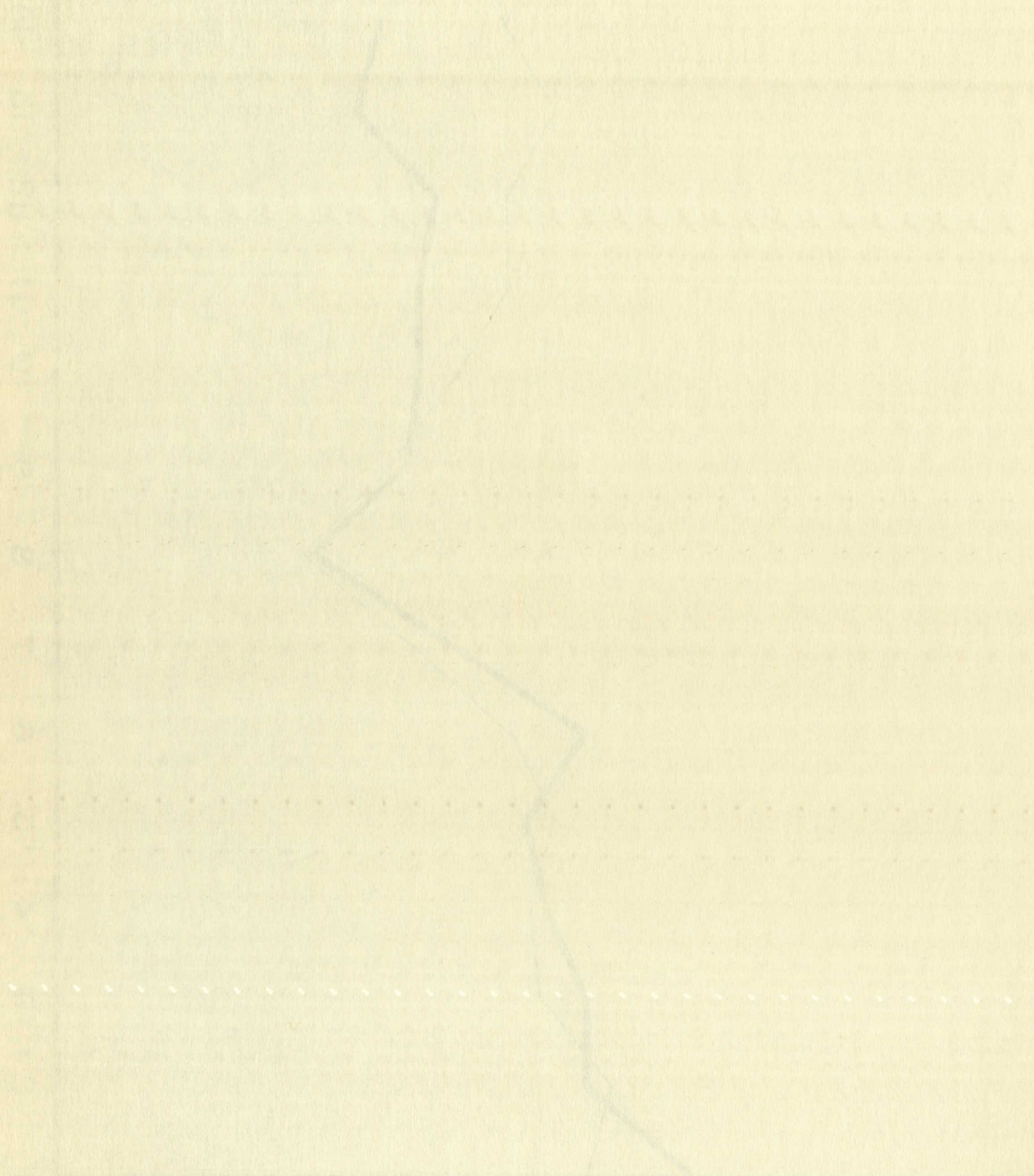


2 VI



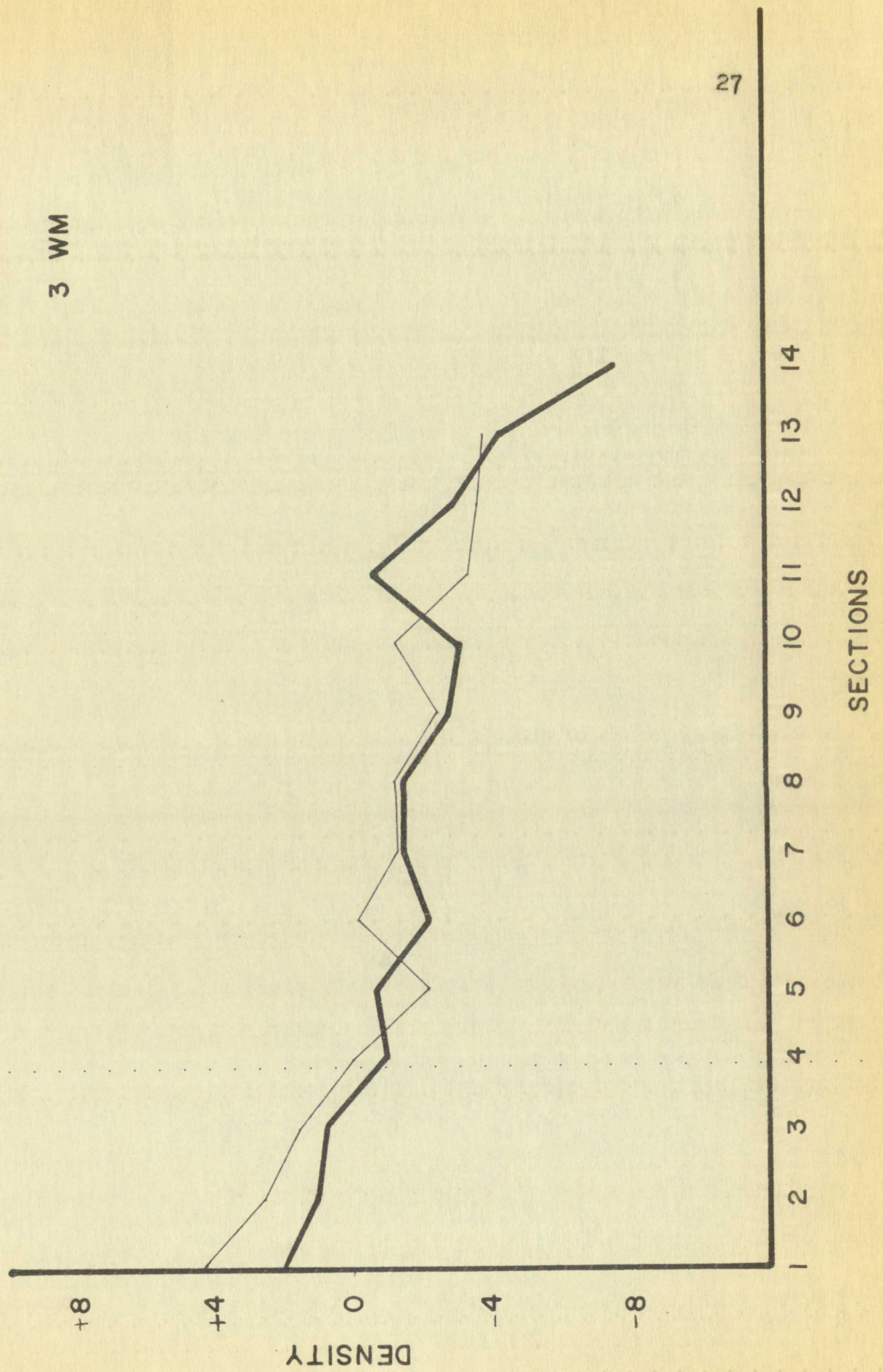


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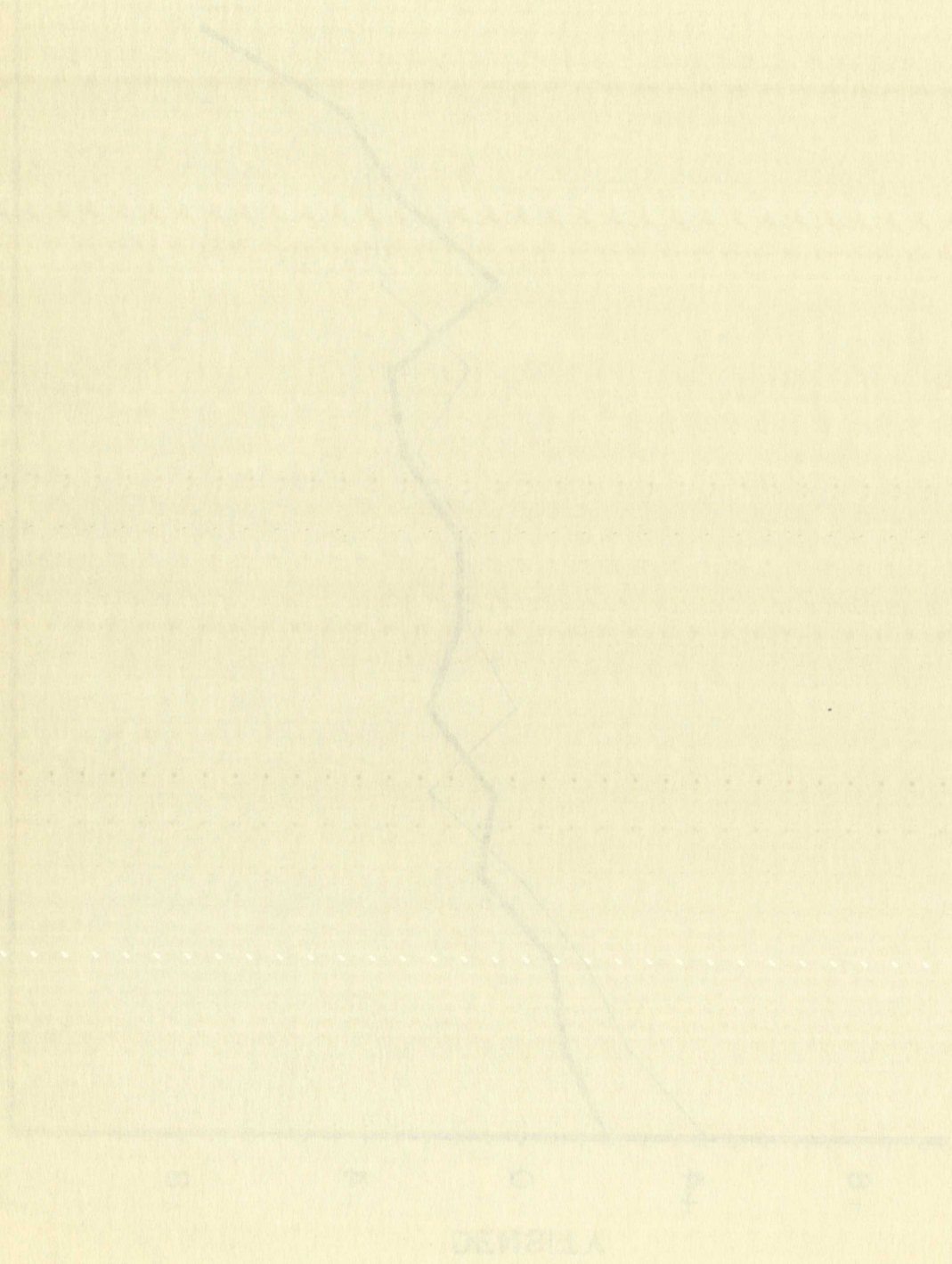


DEPTH



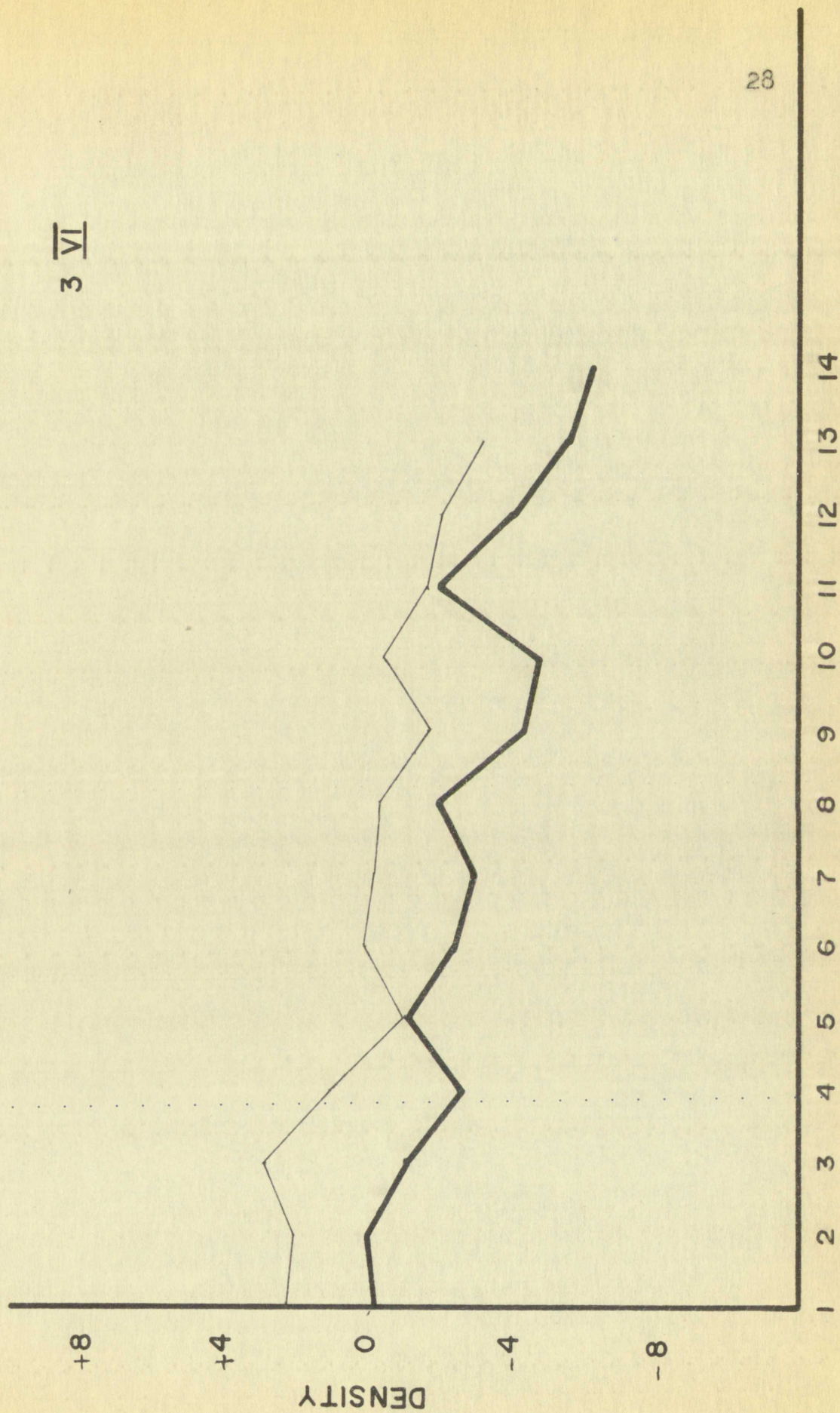








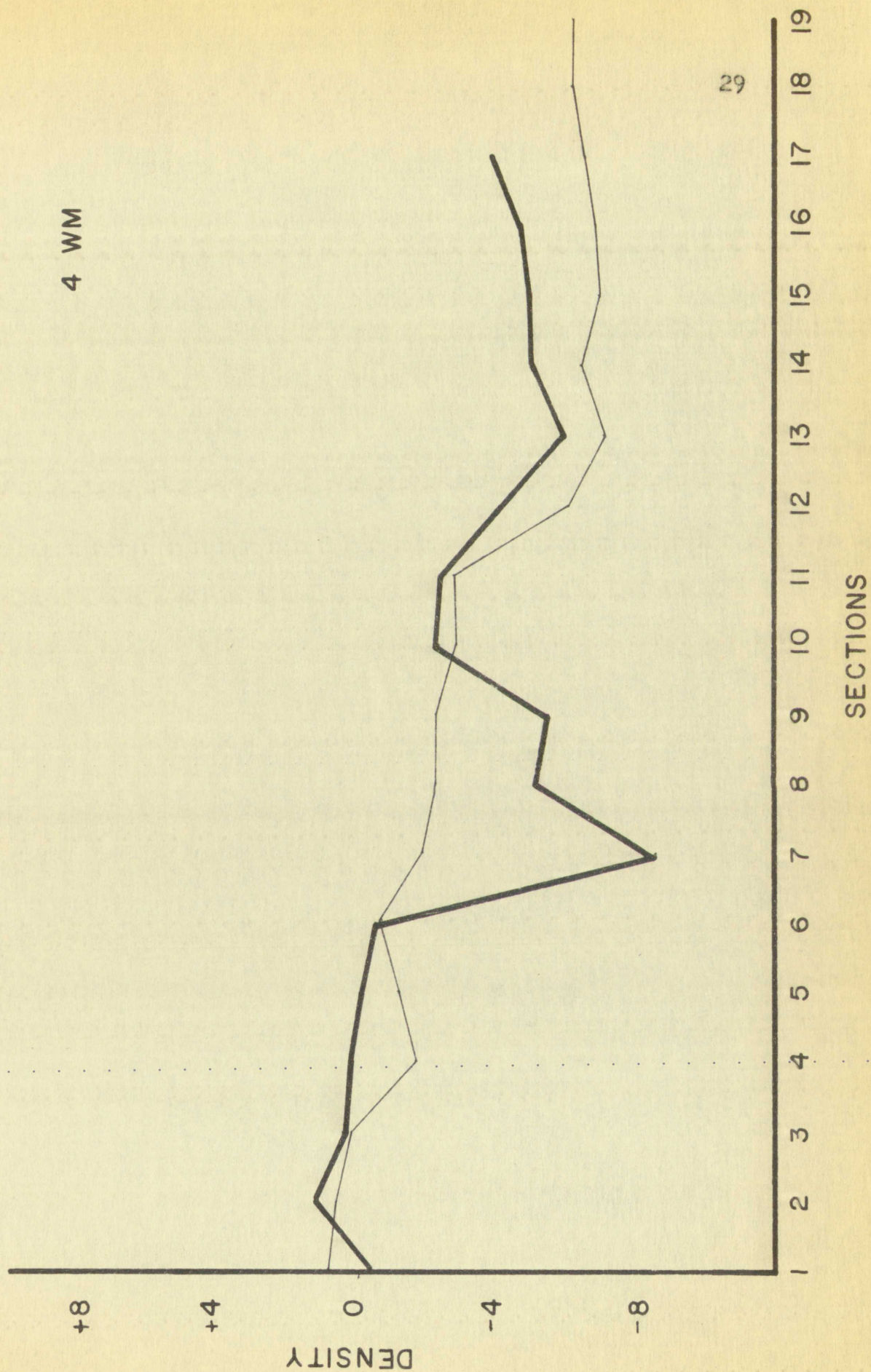
3 VI









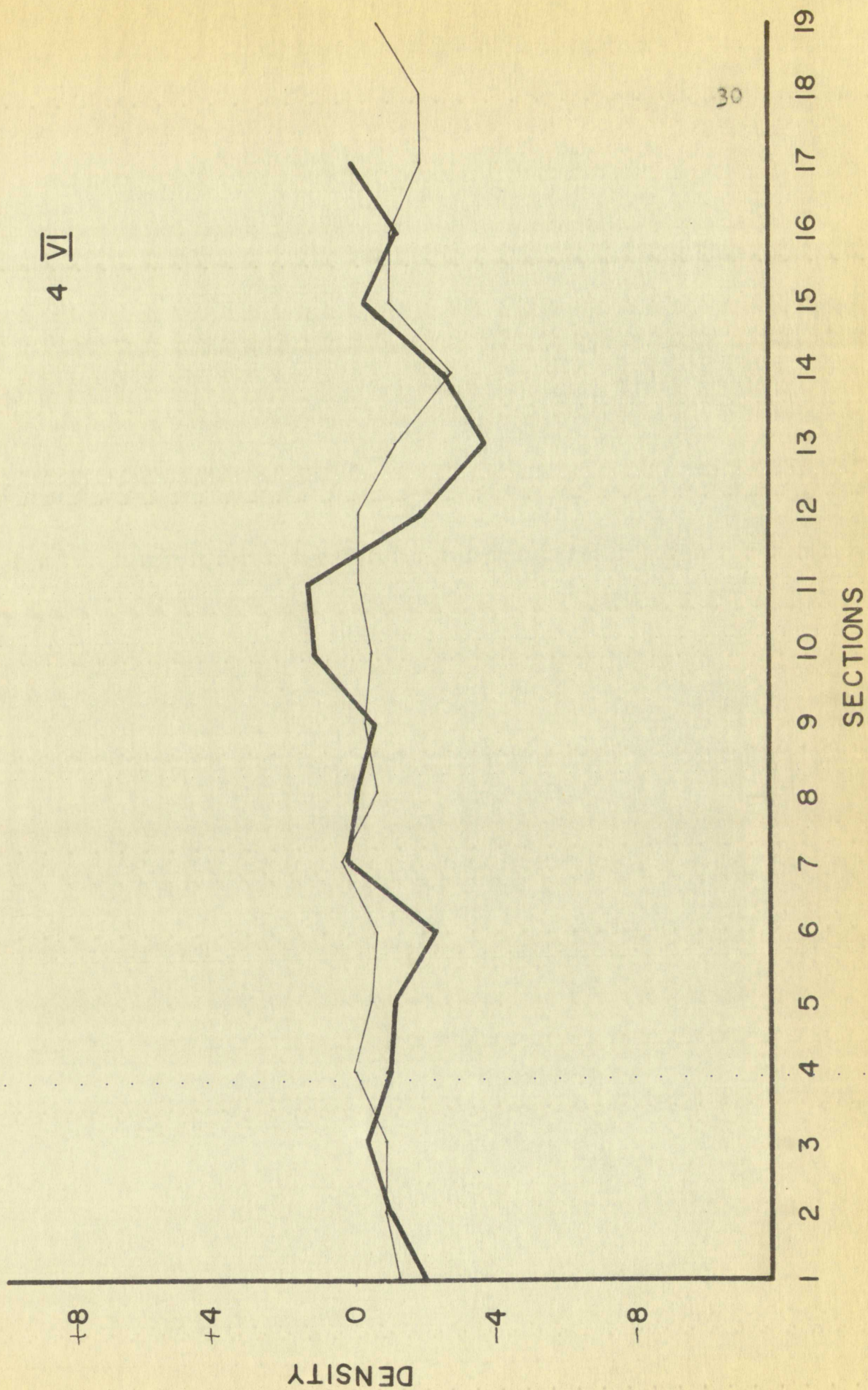








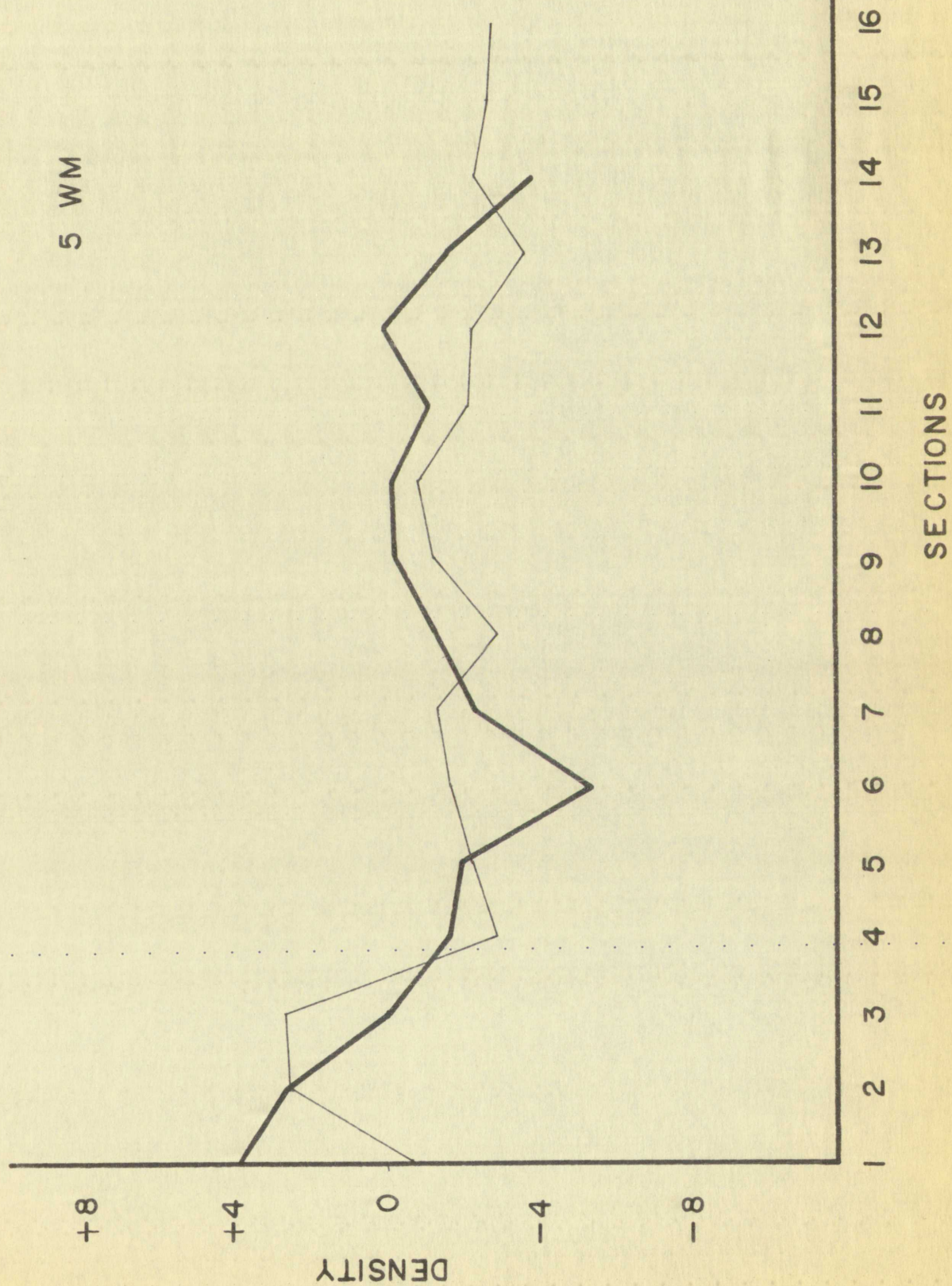
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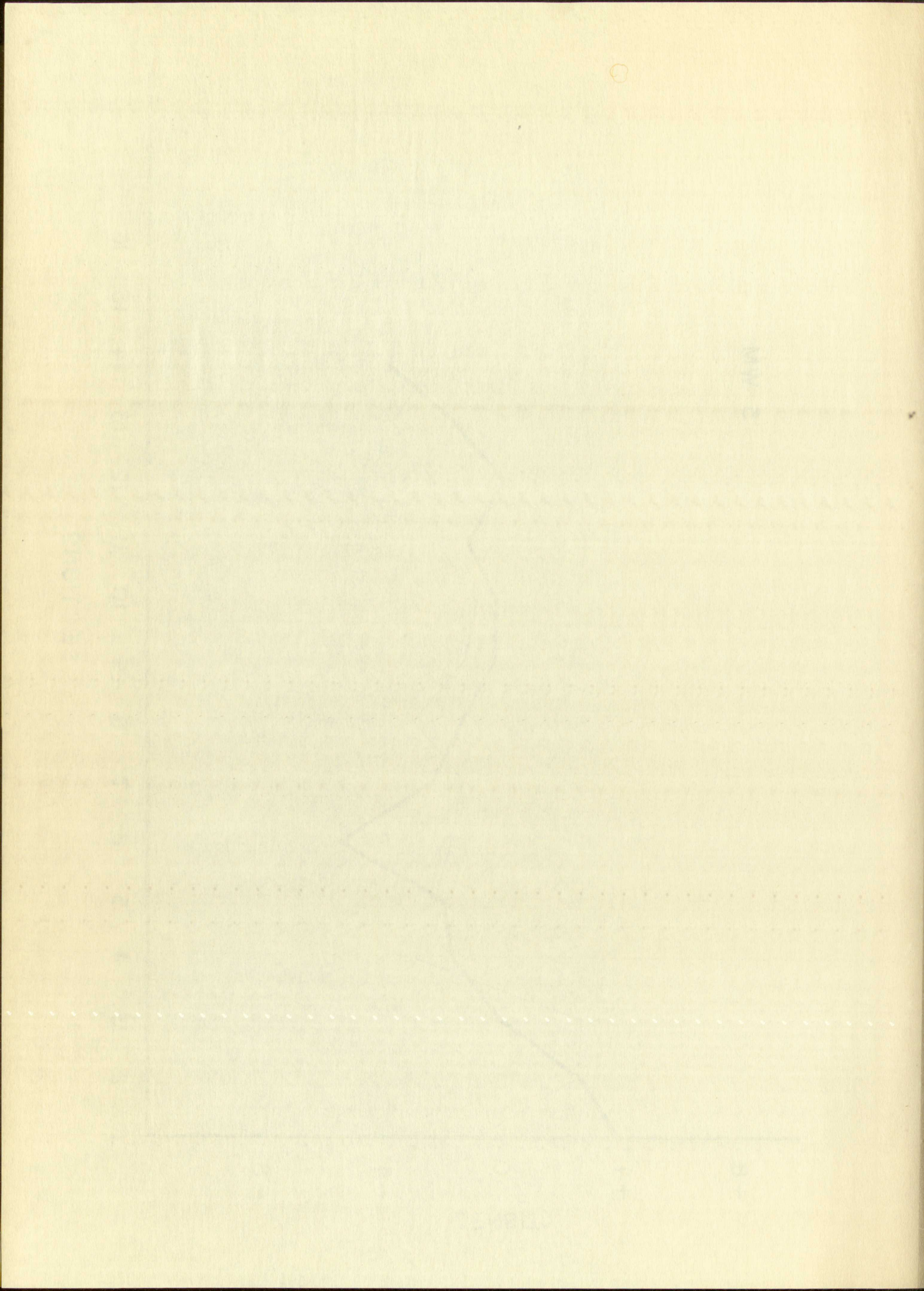






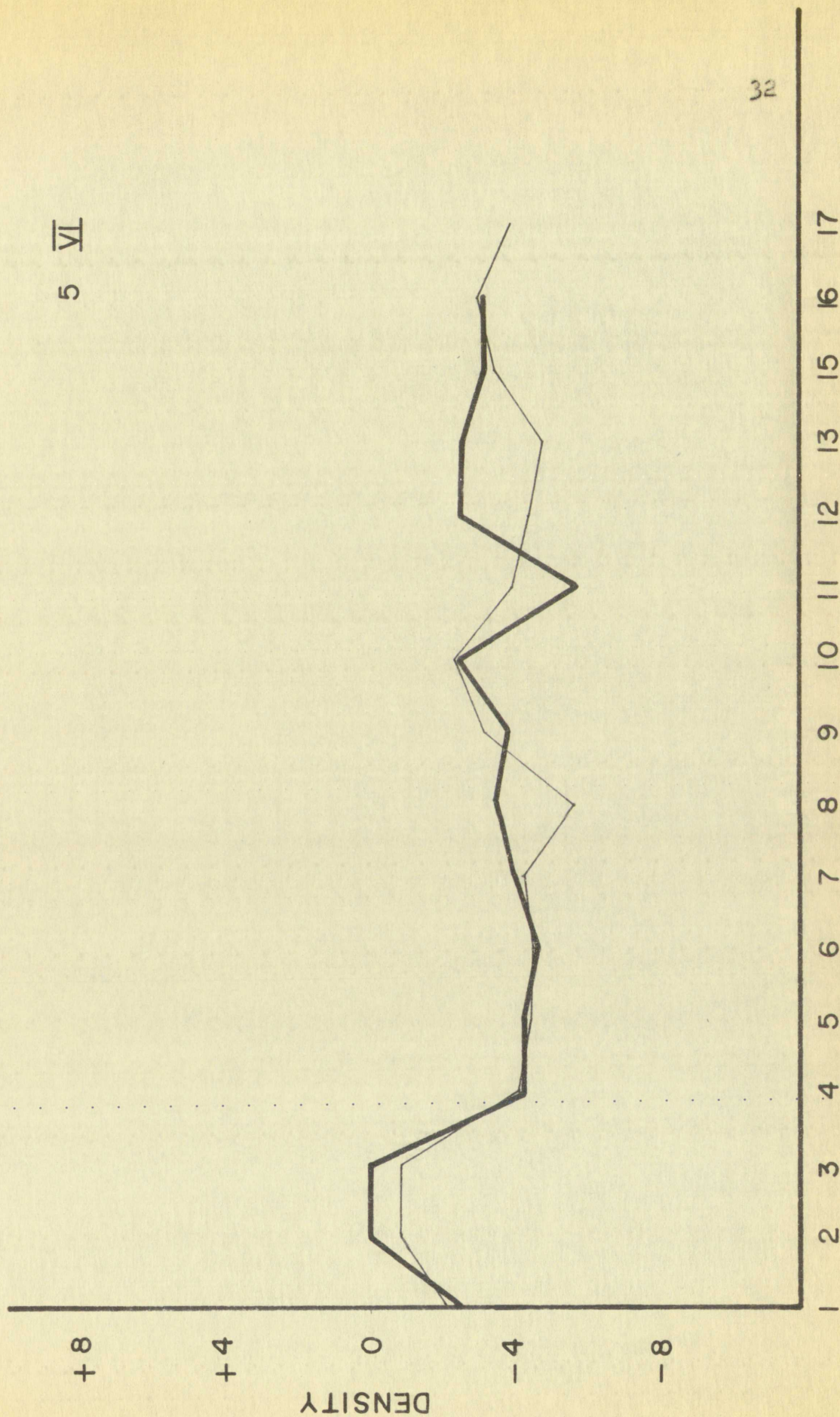








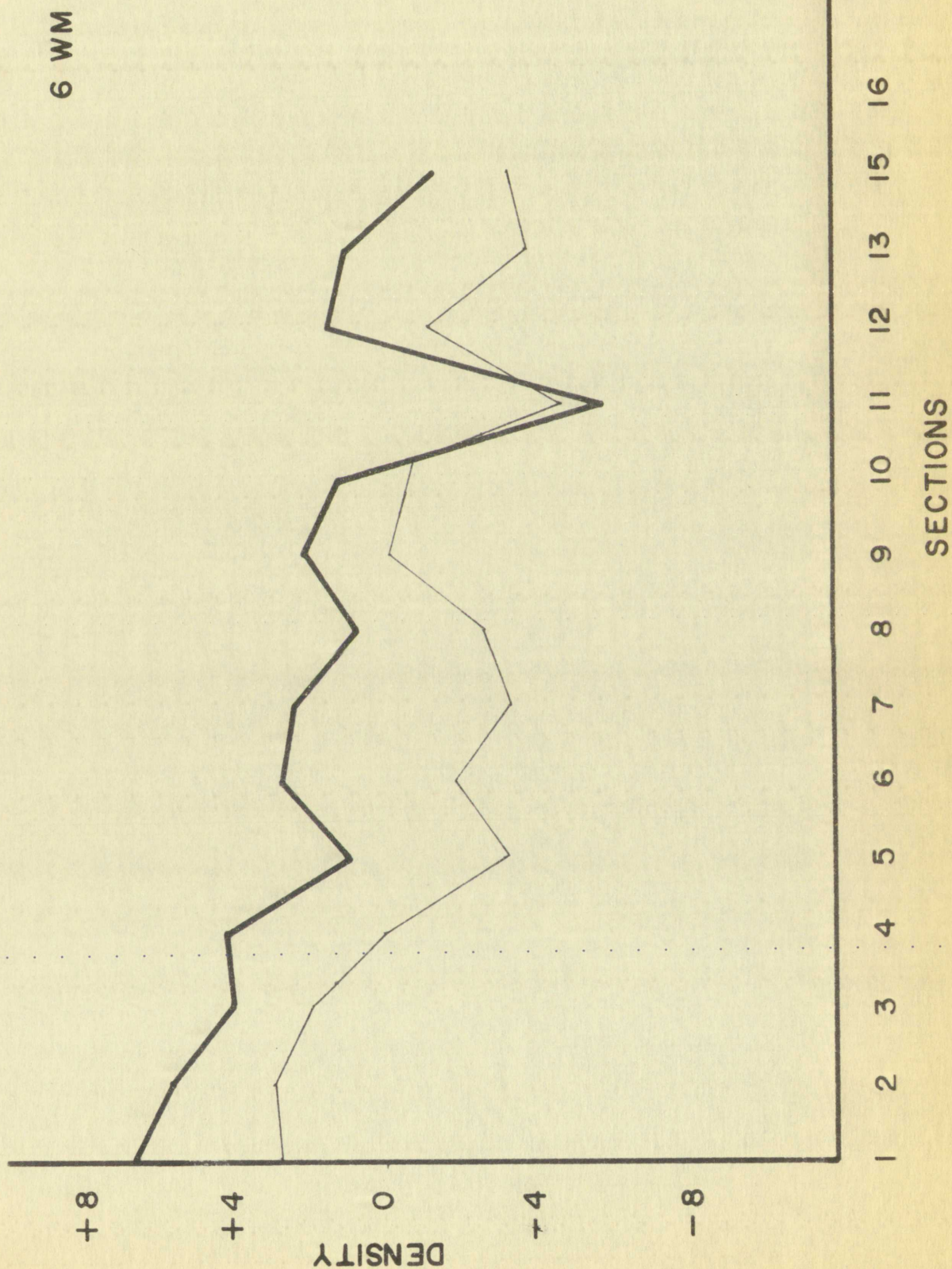
5 VI













6

MM. C.

101101

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0

7

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+

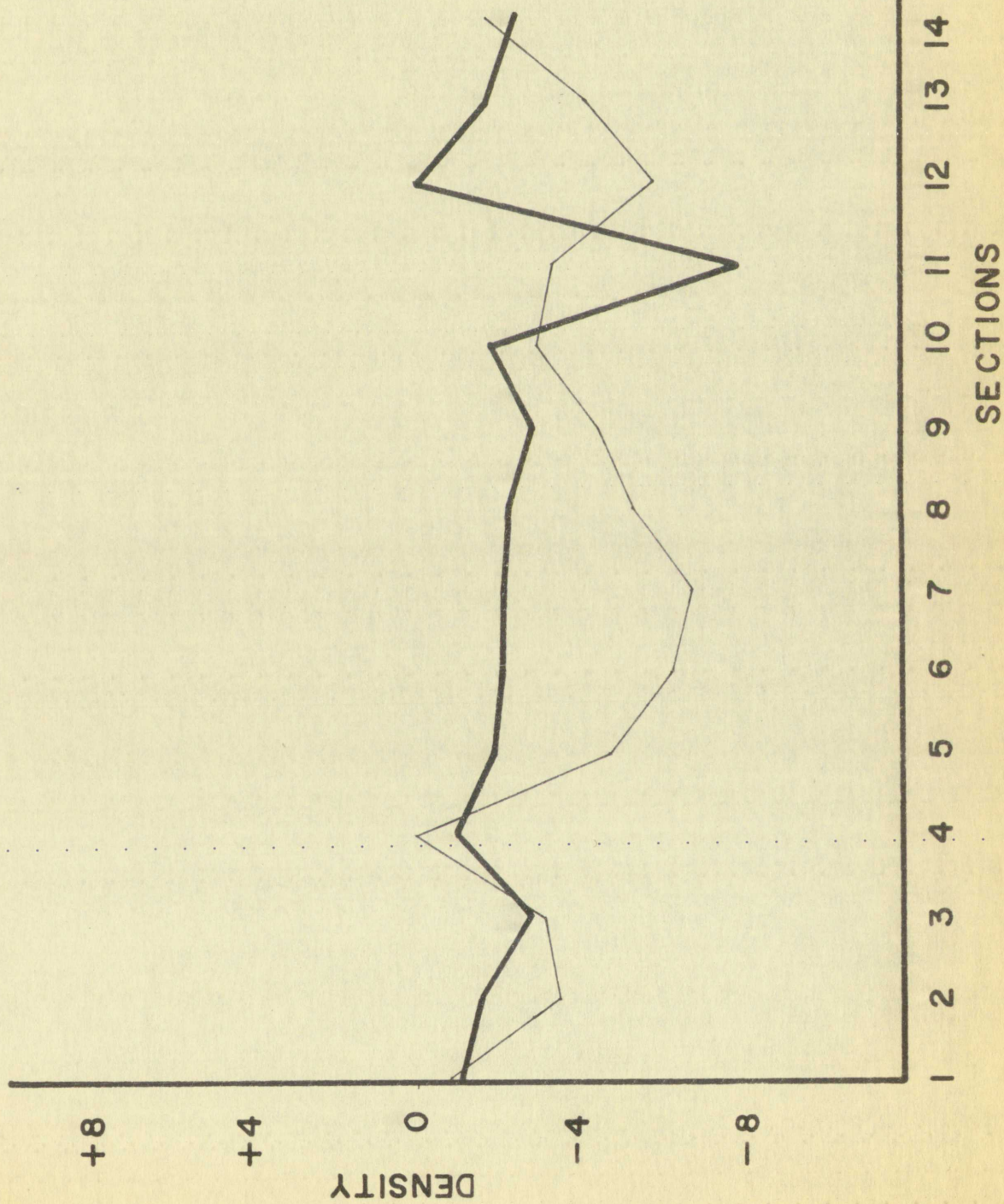
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6 VI

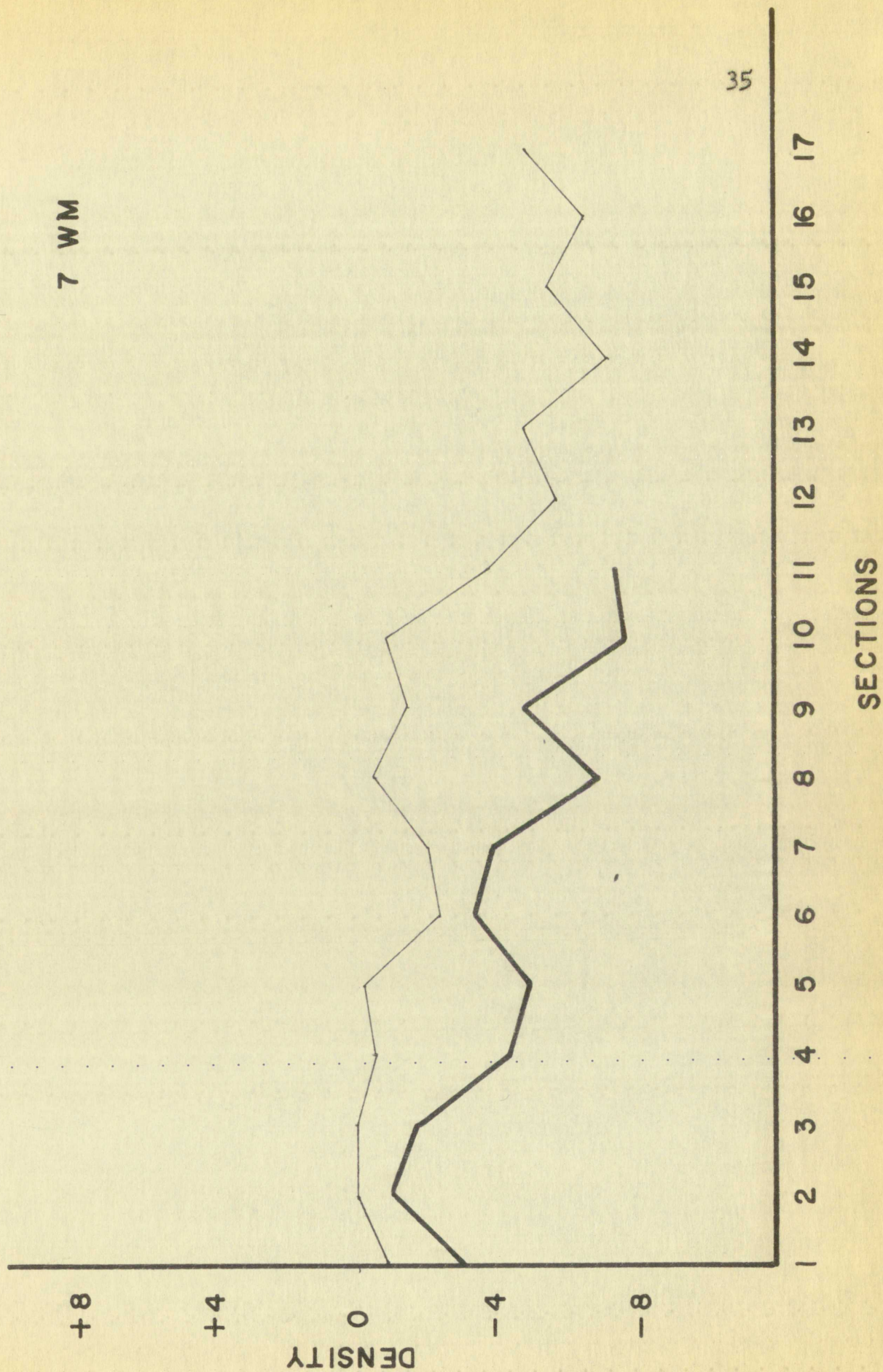




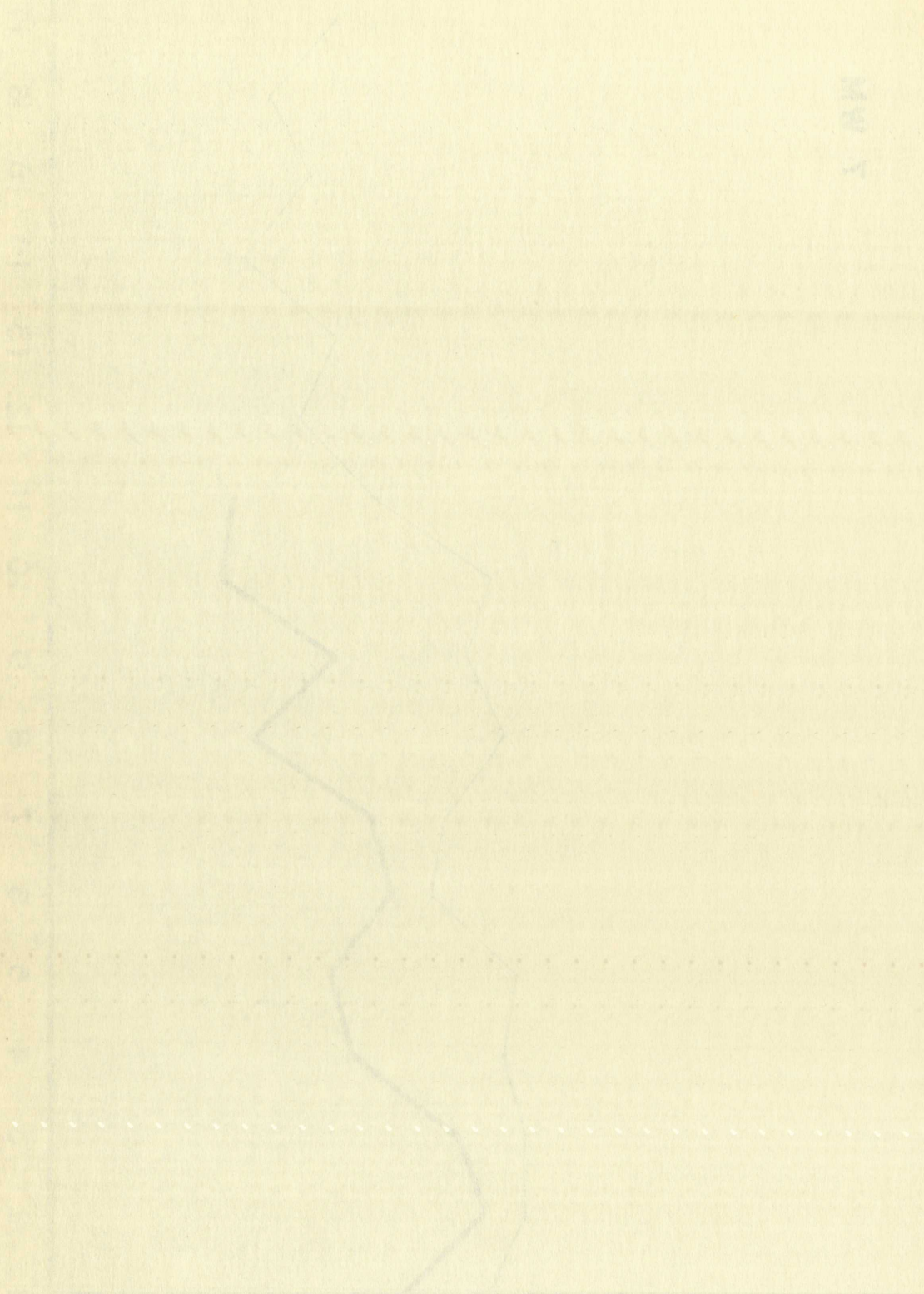
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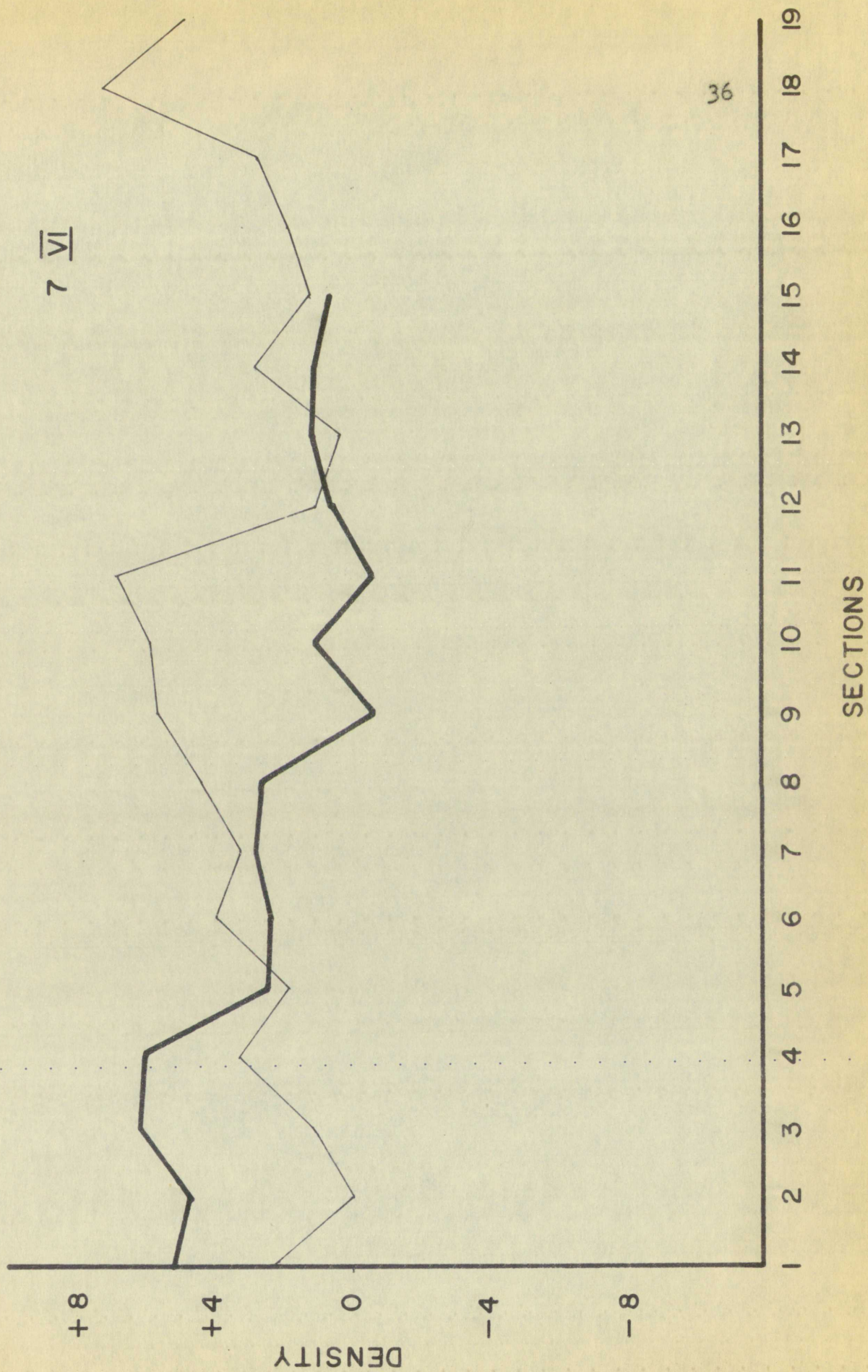




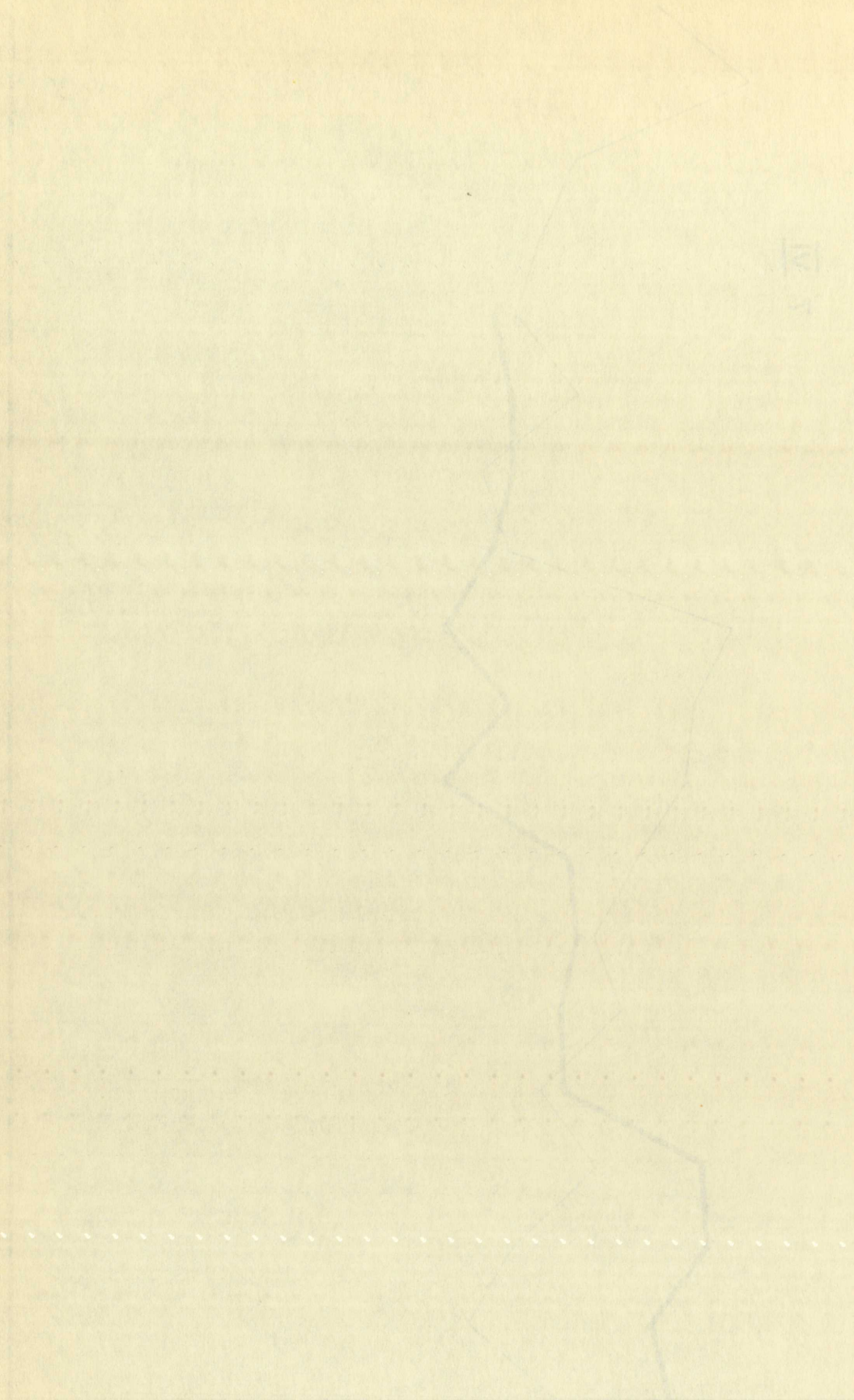


ATLANTA



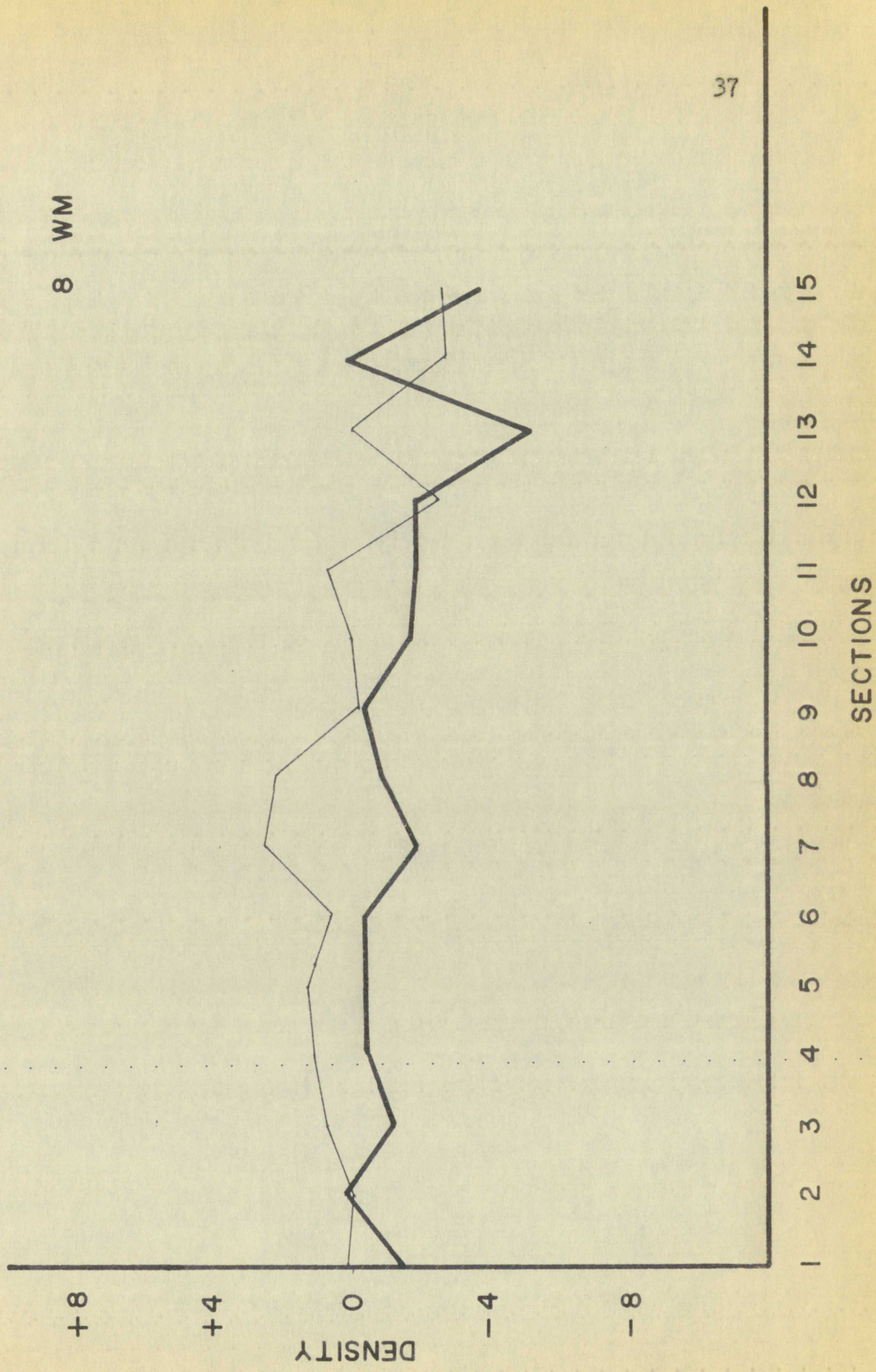




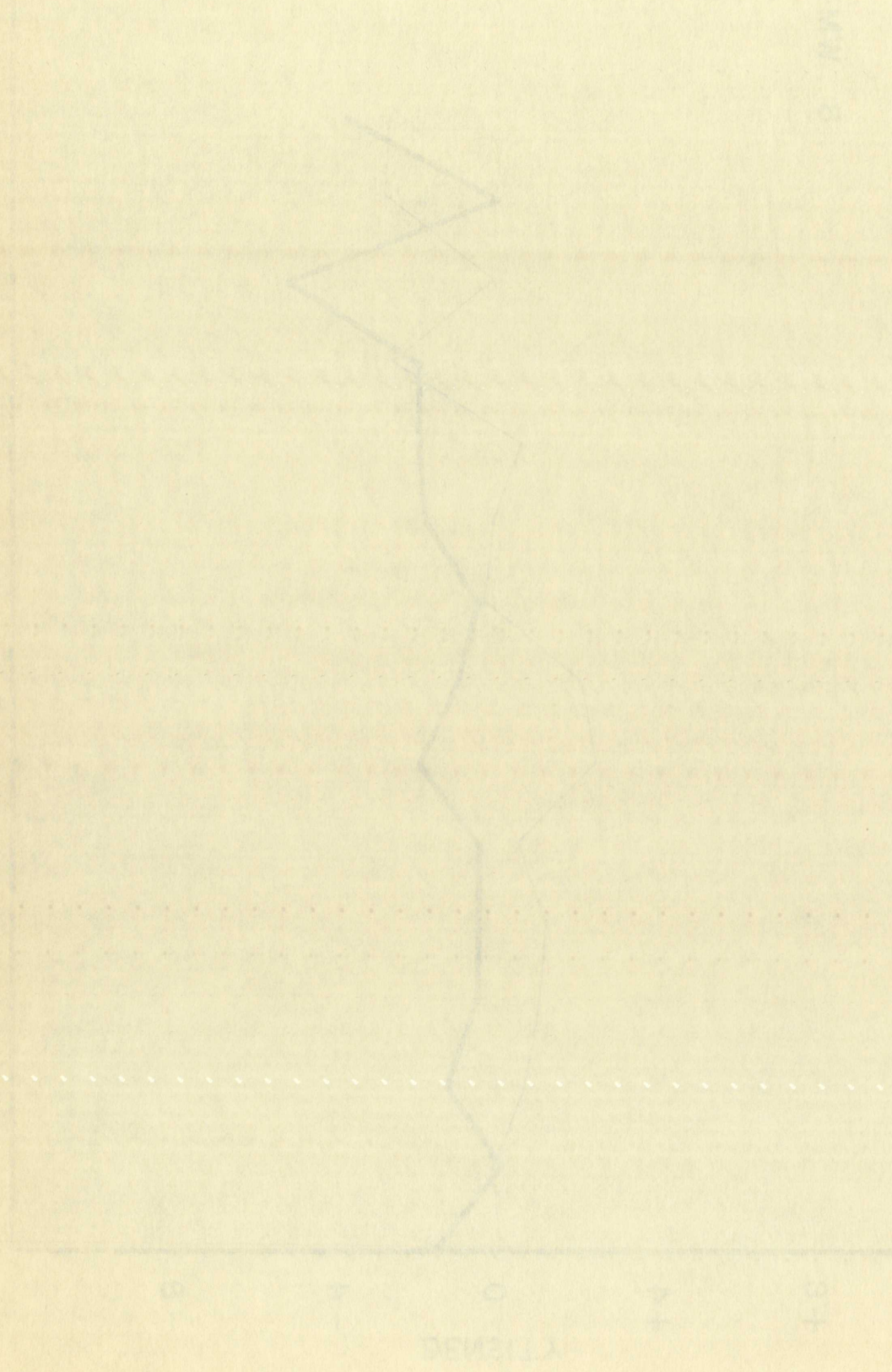


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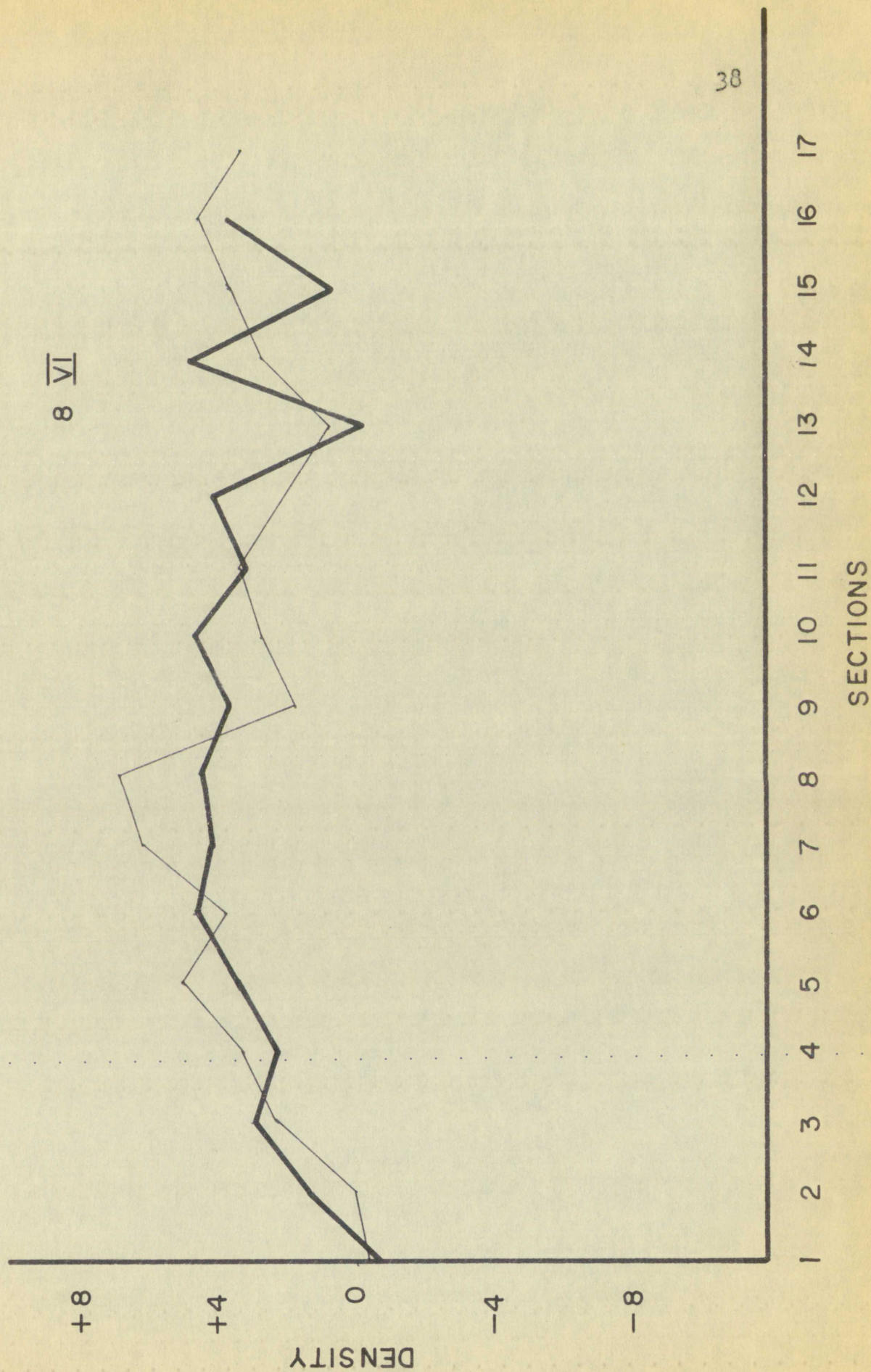




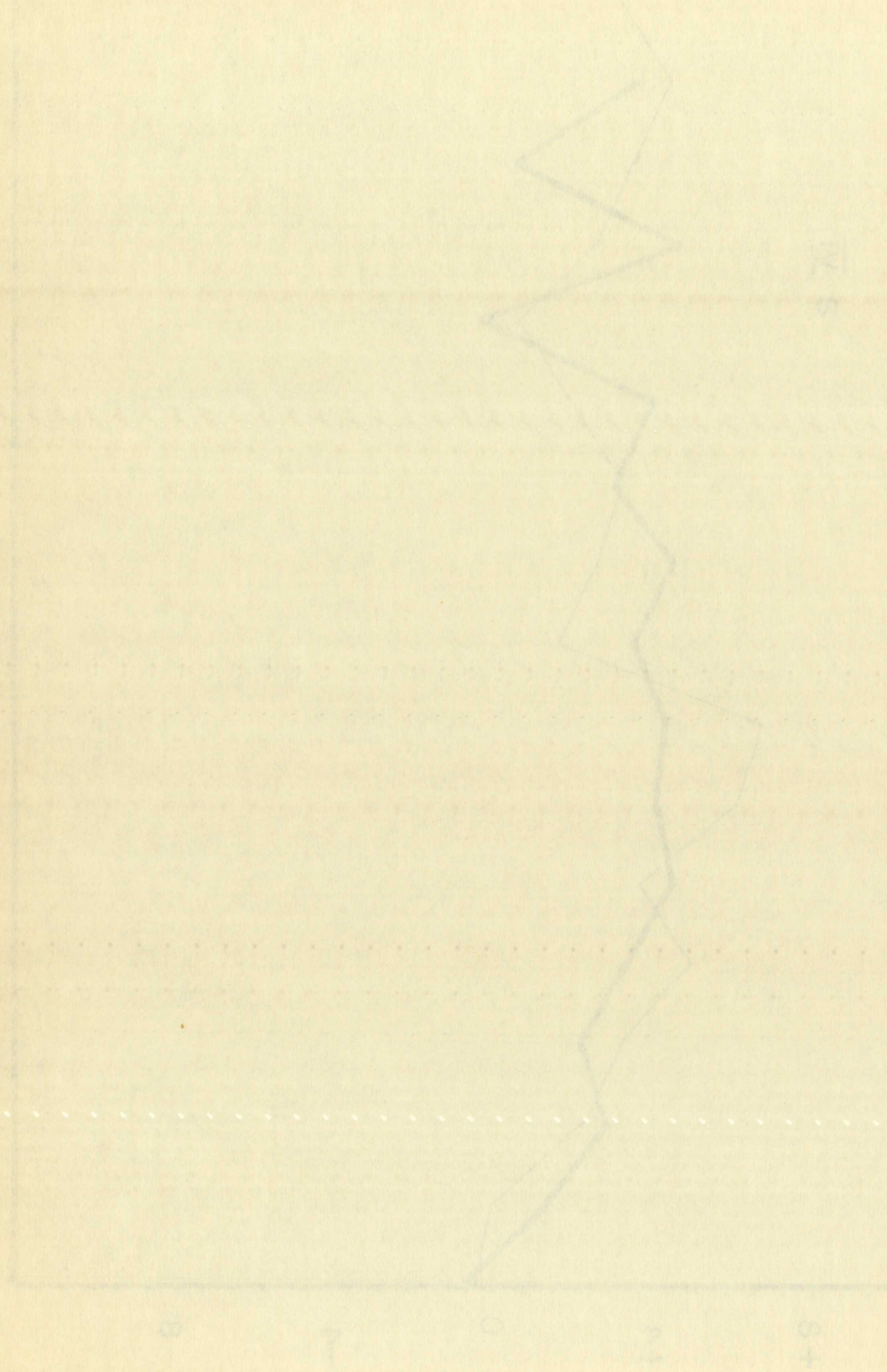






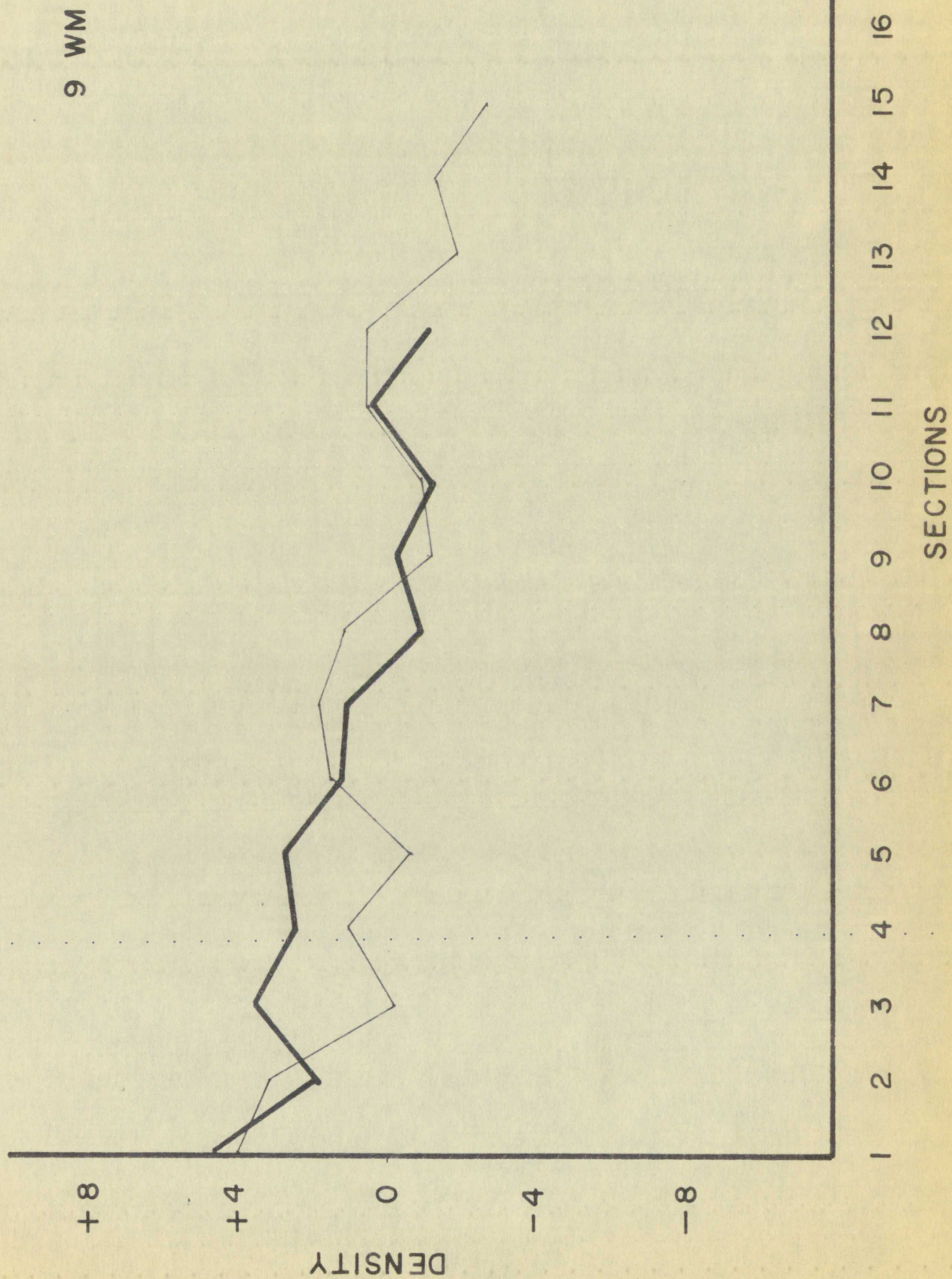






DENSITY

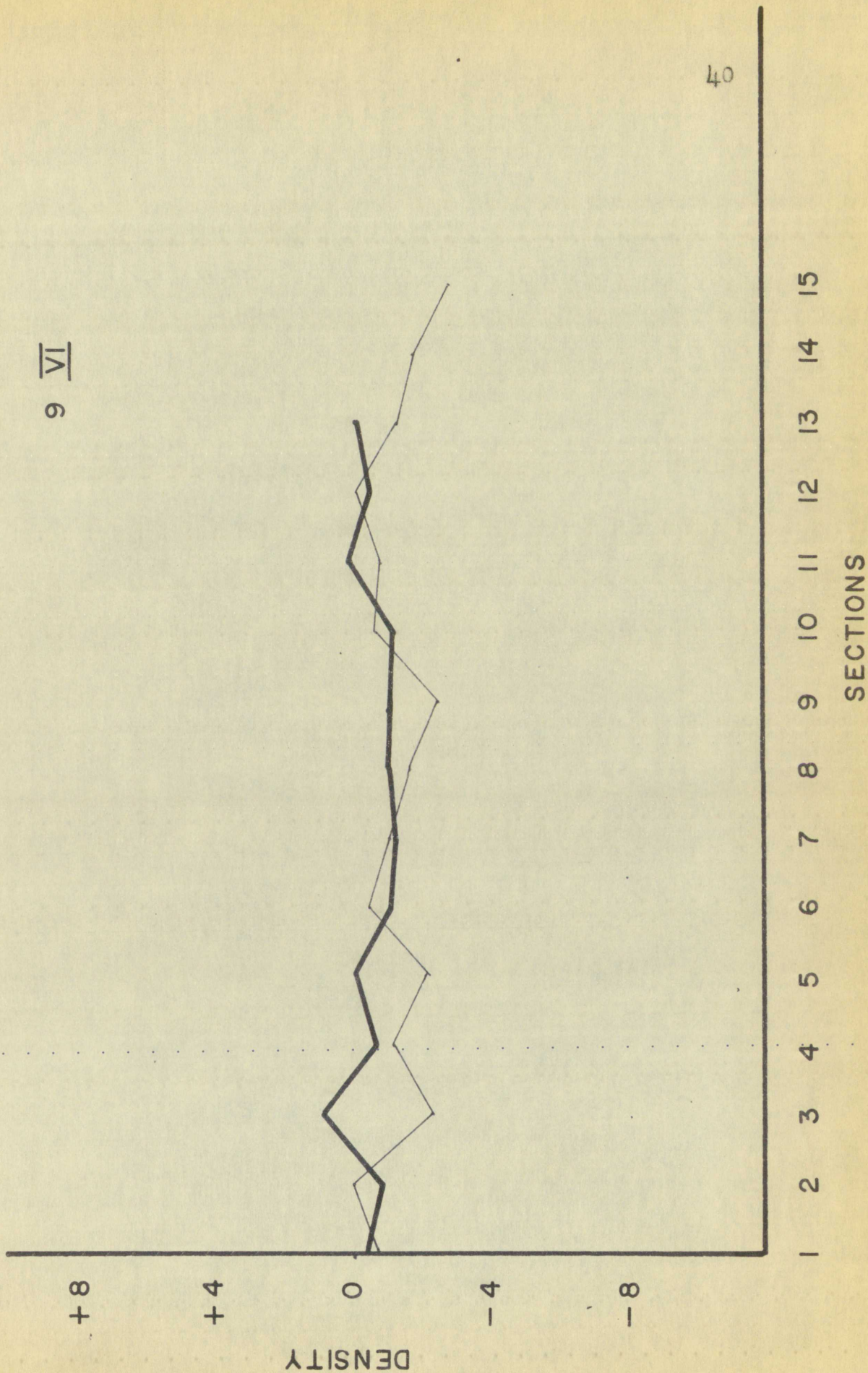








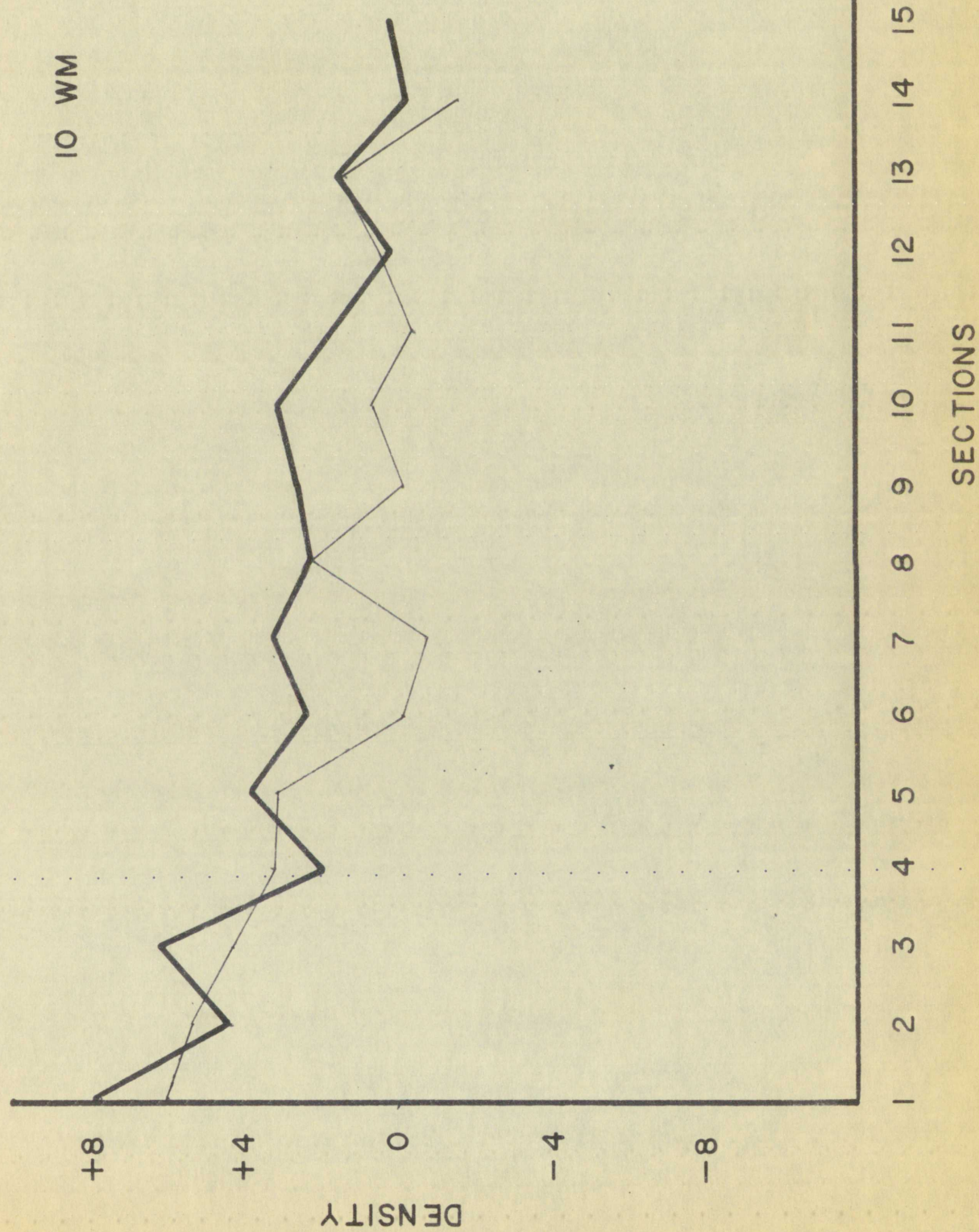




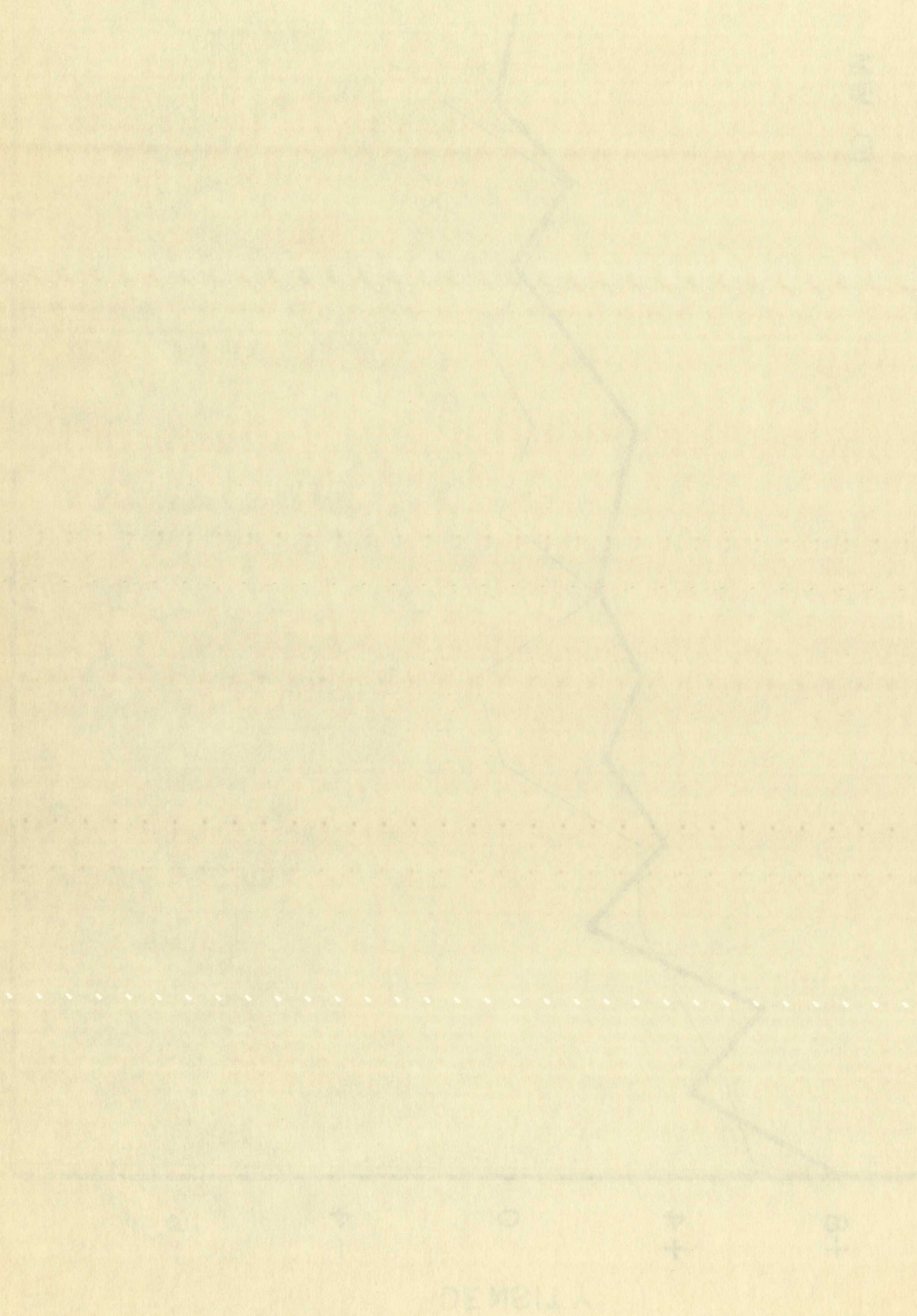






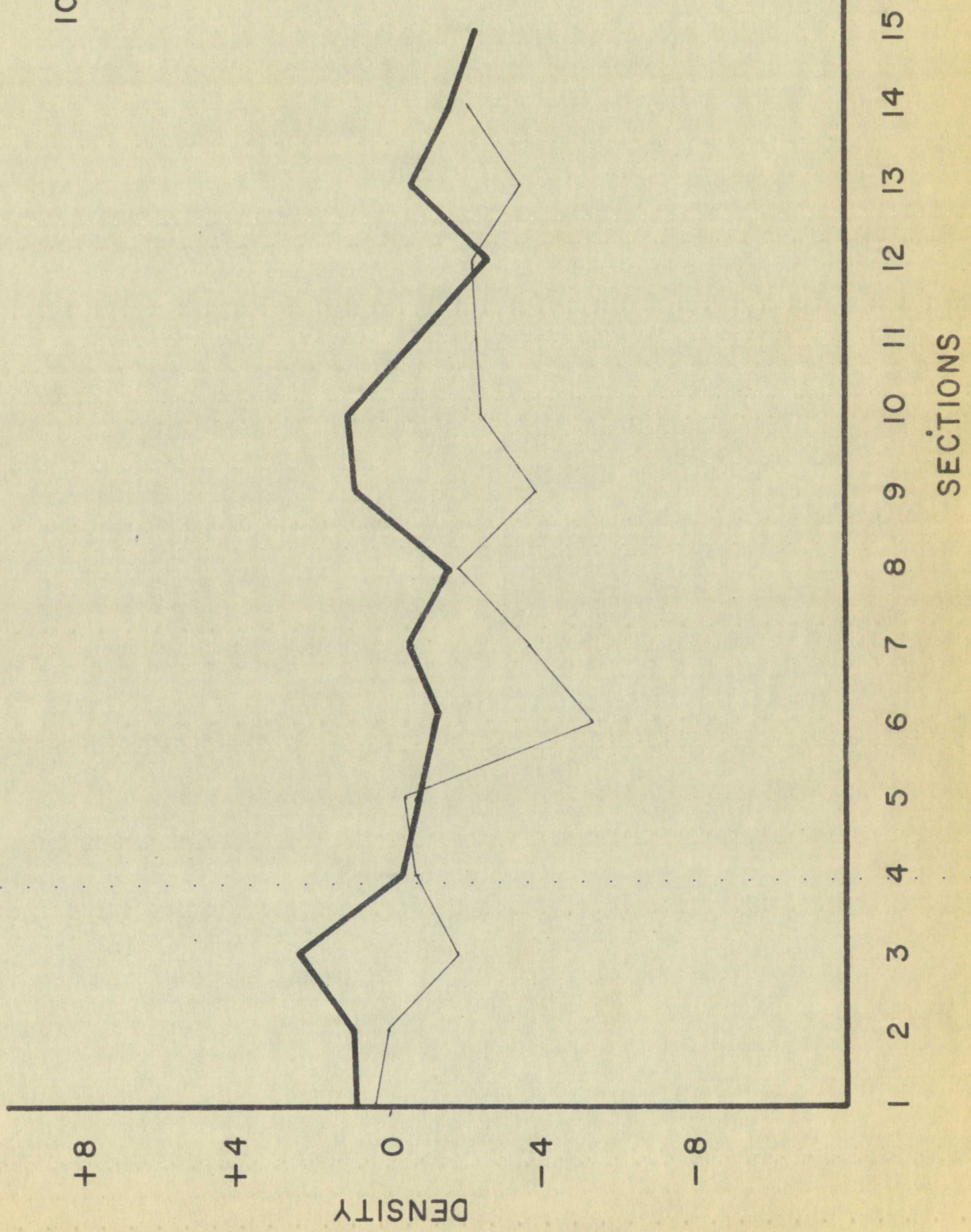






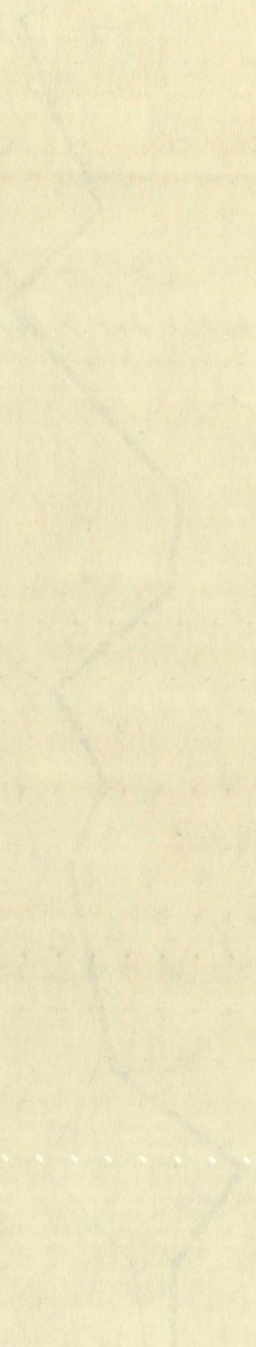


10 VI





100



100



## DISCUSSION

If a relationship between handedness and density of the hemisphere controlling the preferred hand exists, then one should be able to predict the handedness of the animal from the data. Several pilot cases led to the construction of a hypothesis that involved this relationship and specifically stated that the hemisphere controlling the preferred hand would be denser. Consequently, by observation of the graph for any particular animal, one should be able to determine the handedness of this animal. This hypothesis sets up a choice between two hemispheres in each animal, and each of the ten animals is an individual choice situation. There are then ten choice situations, and obviously if prediction of handedness is the key factor in the evaluation of the hypothesis, then the question of chance and its effect upon the results becomes important.

Edwards (1950) cites an experimental design that is quite similar to this problem. Where there are ten sets of items, in each set one being correct and one incorrect, the probability of obtaining all ten sets correctly by chance alone is  $\frac{1}{2}^{10}$  or 1 out of 1024. The probability of having nine sets out of ten is  $10/1,024$ , and two incorrect choices is .055 or slightly below the usual criterion of the 5% level.



## DISCUSSION

It is a relationship between hemisphere and lateralization of the hemisphere controlling the particular response. Then one should be able to predict the hemisphere of the animal from the data. Several little pieces of evidence construction of a hypothesis that involved a relationship ship and specifically stated that the hemisphere controlling the preferred hand would be lateralized. Some quantity by observation of the graph for the particular animal, one should be able to determine the hemisphere of the animal. This hypothesis seems to be a logical extension of hemispheres in each animal, and each of the two hemispheres is an individual choice situation. There are two main choice situations, and obviously a prediction of hemisphere is the key factor in the determination of the hypothesis. Then the question of chance and the level of chance results becomes important.

Edwards (1952) gives an experimental design that is quite similar to this problem. Where there are two items, in each set and being correct and one incorrect, the probability of obtaining all the correct responses by chance alone is  $2^{-n}$  or  $1/2^n$ . For example, if the probability of having nine sets out of 10 correct is  $1/1024$ , and the incorrect choice is  $1/1024$ , the probability of obtaining all the correct responses of the 10 levels is  $1/1024^{10}$ .



Inspection of tables II and III indicates that in the case of layer VI, seven out of ten cases could be predicted; and in the white matter only three cases out of ten could be predicted. Statistically, seven out of ten correct choices gives a probability of .17 which does not meet the usual minimum requirement of .05. Even if the hypothesis were reversed, that is to say that the lightest hemisphere had predictive value, the white matter data would also give a P of .17 which is at a chance level. Consequently the hypothesis which states that density of a hemisphere (in the white matter and layer VI as measured in this experiment) has predictive value in relation to handedness is not supported.

After this general negative result, the graphs were examined in terms of a second hypothesis, namely that the pattern of the graphs might be related to handedness. This approach also turns out to be unfruitful. Many pattern hypotheses were developed in terms of results on one or two graphs, but broke down when subjected to more thorough examination.

Actually, in some of the cases, the graphs could not be classified by inspection and it was necessary to compute means, obviously not significant, in order to classify for density.



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Table II -- Predictive value of WM data

<u>Rat No.</u>	<u>Dense Hemisphere (WM)</u>	<u>Preferred Hand</u>	<u>Prediction</u>
1	R	R	-
2	L	R	+
3	L	R	+
4	R	R	-
5	R	R	-
6	L	L	-
7	L	L	-
8	R	L	+
9	L	L	-
10	L	L	-

3/10 correct



Table II -- Predictive value of WM data

Rat No.	Danese Hemisphere (WM)	Preferred Hand	Prediction
1	R	R	-
2	L	R	-
3	L	R	+
4	R	R	-
5	R	R	-
6	L	L	-
7	L	L	-
8	R	L	+
9	L	L	-
10	L	L	-

3/10 correct



Table III -- Predictive value of layer VI data

<u>Rat No.</u>	<u>Dense Hemisphere (VI)</u>	<u>Preferred Hand</u>	<u>Prediction</u>
1	L	R	+
2	L	R	+
3	L	R	+
4	L	R	+
5	R	R	-
6	L	L	-
7	R	L	+
8	R	L	+
9	R	L	+
10	L	L	-

7/10 correct



Table III -- Predictive value of lower VI tests

Rat No.	Dance Hemisphere (VI)	Predicted Hand	Direction
1	L	R	+
2	L	R	+
3	L	R	+
4	L	R	+
5	R	R	-
6	L	L	-
7	R	L	+
8	R	R	+
9	R	L	-
10	L	L	-

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Consequently, it can be concluded that this first step in the examination of cortical tissue for structural changes has yielded a negative result. At the same time it should be said that this step is a first but simple approach to the problem. With the present technique two discrete readings have been taken from two arbitrarily selected spots in the cortex. The point is that if all the lamination of the cortex had been examined with this particular stain or perhaps with some other stain the results might not have been negative, and at the present time we have developed a combination of instrumentation that will achieve this goal of laminar examination. The cortex is continuously scanned by the light beam and this information is fed to an oscilloscope. The result is a density lamination curve such as Figure 2.



Consequently, it can be concluded that the first step in the examination of cortical tissue for changes has yielded a negative result. As the author it should be said that this was the first step in approach to the problem. With the present knowledge discrete readings have been taken from two randomly selected spots in the cortex. The point is that the lamination of the cortex has been examined with particular stain or perhaps with some other. In some results might not have been negative, and in some times we have developed a combination of techniques that will achieve this goal of laminar examination. The cortex is continuously scanned by the light beam and the information is fed to an oscilloscope. The results of density lamination curve appear as follows:



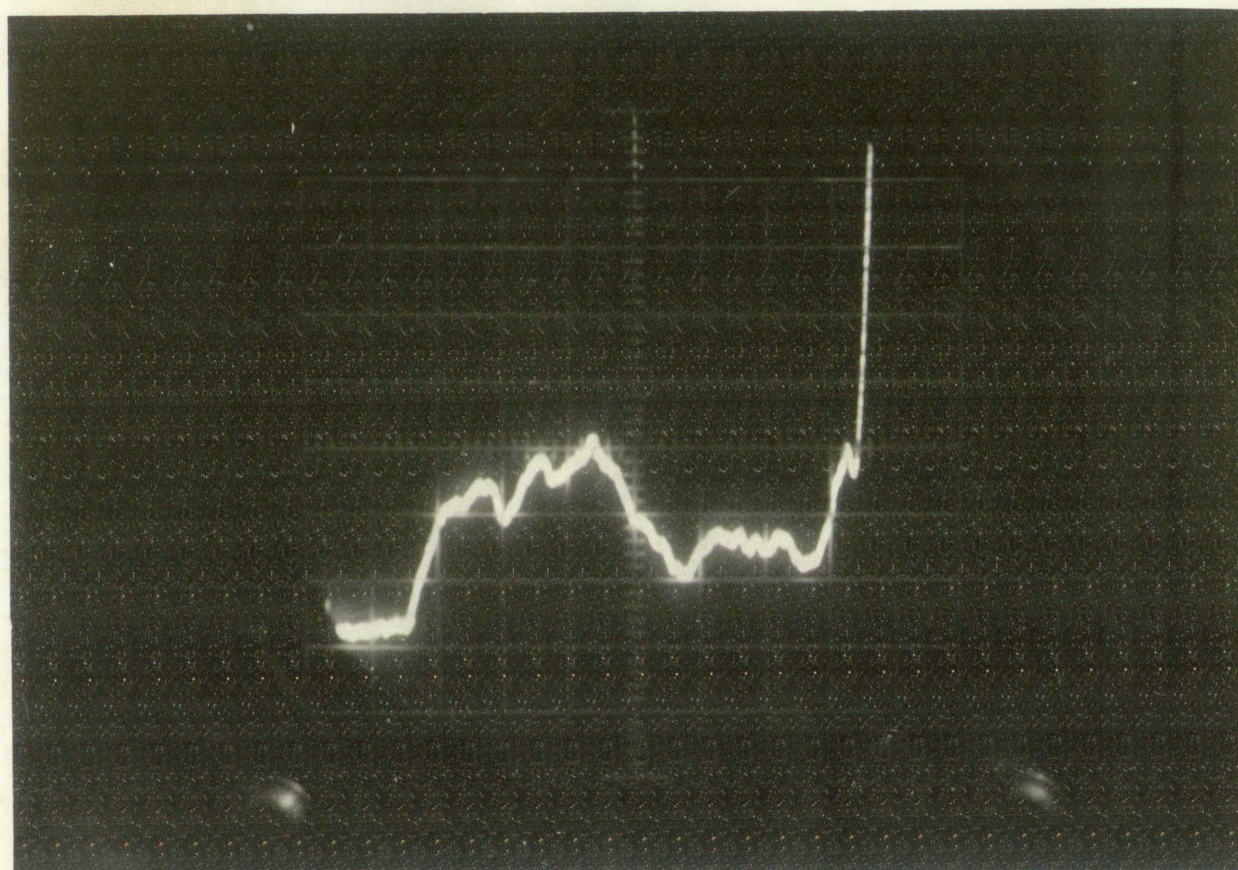


Figure 2 -- Lamination Curve



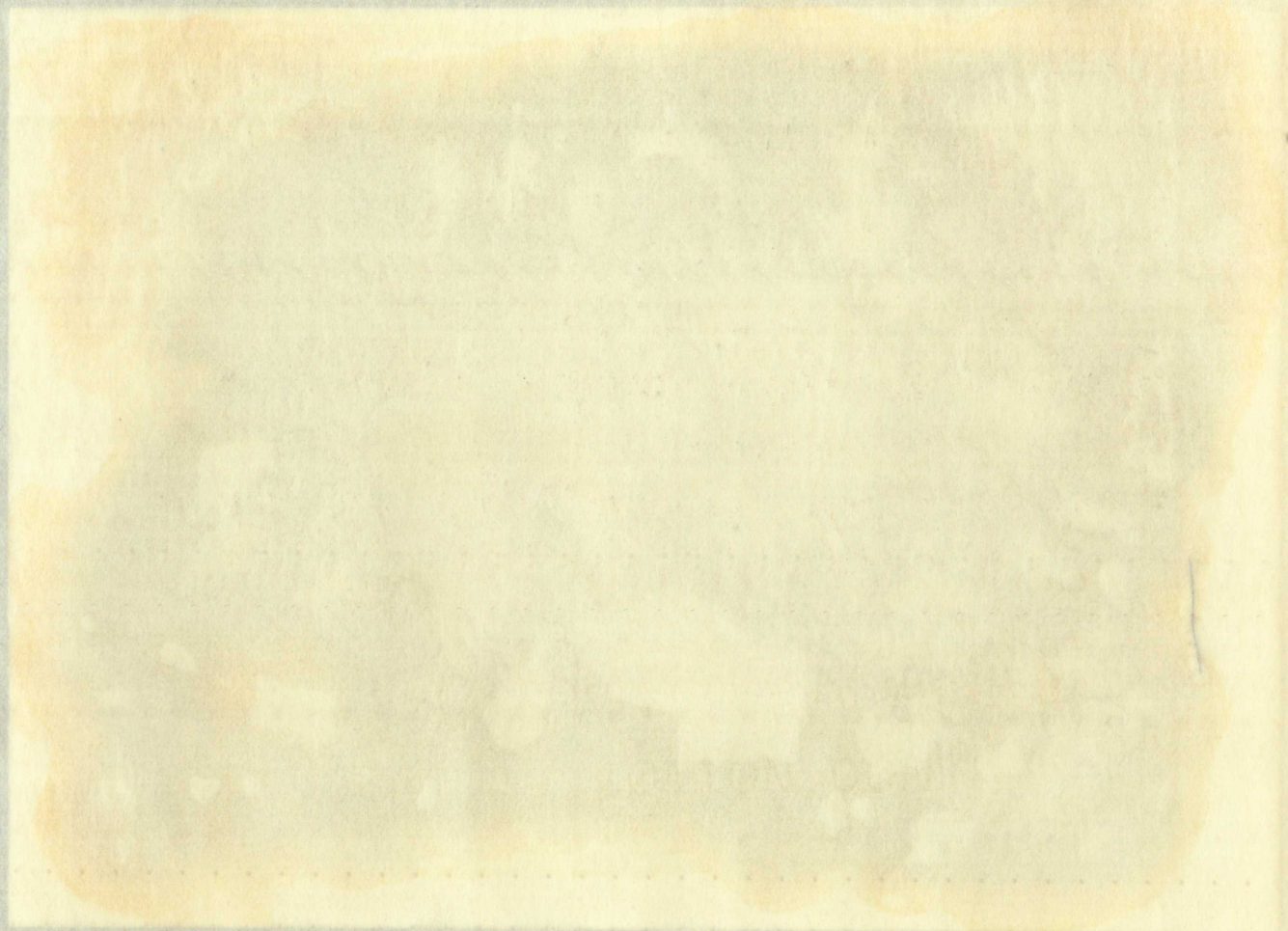


Figure 2 -- Laminated Glass



This projected approach is more complex than the present one and will be, perhaps, more fruitful. The simple approach was tried first because if positive results had been found the second approach would have been unnecessarily complex.

Another advantage in the second approach is that electrical stimulation will be used to locate the handedness area, and electrical desiccation will be used to produce a small lesion that will serve as a mark. After processing the brain, the mark will appear on those slides designated by electrical stimulation and lamination scanning will be performed under those marks. This approach has already been established as experimentally feasible. (Earp, 1959)

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This projected approach is most likely to be the present one and will be, perhaps, the first of the simple approach was tried first because of past experience had been found the second approach would have been much easier to apply.

Another advantage in the second approach is that electrical stimulation will be used to locate the motor area, and electrical stimulation will be used to produce a small lesion that will serve as a marker. In processing the brain, the area will appear in those slides designated by electrical stimulation and stimulation scanning will be performed with these markers. This approach has already been established as experimentally feasible. (Barry, 1959)



## SUMMARY AND CONCLUSIONS

Ten rats were tested for preferential handedness, their brains removed, and the area in each hemisphere between white matter and caudate nucleus sectioned at  $40\mu$ . These sections were stained by the Kluver-Barrera technique. The sections were investigated photometrically by measuring optical density in the white matter and layer VI of the cortical area concerned with handedness. The results of this investigation reveal no relation between density of these cortical areas and preferential handedness. Consequently, the hypothesis under test, that is, that the white matter and layer VI of the preferred hemisphere impede more light than those of the hemisphere controlling the non-preferred hand, is not supported.

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## SUMMARY AND CONCLUSIONS

Ten rats were tested for preferential handedness. Their brains removed, and the area in each hemisphere between white matter and cerebral nucleus sectioned at 40  $\mu$ . These sections were stained by the Nissl-Barrett technique. The sections were investigated photomicrographically by measuring optical density in the white matter and layer VI of the cortical area concerned with handedness. The results of this investigation reveal no relation between density of these cortical areas and preferential handedness. Consequently, the hypothesis under test, that is, that the white matter and layer VI of the preferred hemisphere imbeds more light than those of the hemisphere controlling the non-preferred hand, is not supported.



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REVERSE



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APPENDIX



APPENDIX

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## APPENDIX

### KLUVER-BARRERA STAINING METHOD

- A. Make up a 0.1% solution of Luxol Fast Blue MBS by dissolving 1 gram of the substance in 1000 cc. of 95% alcohol. Add 5 cc. of 10% acetic acid. Filter before using. (This solution is very stable and may be used even after one year.)
- B. Make up a 0.1% aqueous solution of Grubler's Cresyl-echtviolett or, if not available, a 0.25% solution of Coleman and Bell's cresyl violet. Before using, add 5 drops of 10% acetic acid to every 30 cc. of solution and filter. When using the Coleman and Bell product, heat solution before filtering.

#### Method for Frozen Sections

1. Cut sections at 40 microns and place in distilled water.
2. Immerse in 70% alcohol for 10 to 15 minutes.
3. Stain overnight (16 to 24 hours) in solution A at 40 degrees C. (For example, for staining 4 sections of the brain stem of a monkey, 20-25 cc. of solution A should be used and then discarded.)
4. Immerse in 95% alcohol and wash off excess stain.
5. Wash in distilled water. (Prolonged washing in water will not affect the staining.)



# APPENDIX

## KLUVER-BARRERA STAINING METHOD

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- B. Make up a 0.1% aqueous solution of Oran's Cresyl-euchrysolite or, if not available, a 0.25% solution of Coleman and Bell's cresyl violet. Before using, add 5 drops of 10% acetic acid to every 50 cc. of solution and filter. When using the Coleman and Bell product, heat solution before filtering.

### Method for Frozen Sections

1. Cut sections at 40 microns and place in distilled water.
2. Immerse in 70% alcohol for 10 to 15 minutes.
3. Stain overnight (16 to 24 hours) in solution A at 40 degrees C. (For example, for staining 4 sections of the brain stem of a monkey, 20-25 cc. of solution A should be used and then discarded.)
4. Immerse in 95% alcohol and wash off excess stain.
5. Wash in distilled water. (Prolonged washing in water will not affect the staining.)



6. Begin differentiation by quick immersion in a 0.005% aqueous lithium carbonate solution.
7. Continue differentiation in several changes of 70% alcohol until gray and white matter can be distinguished. Care should be taken not to overdifferentiate.
8. Wash in distilled water.
9. Finish the differentiation by briefly rinsing in 0.05% lithium carbonate and then putting through several changes of 70% alcohol until there is a sharp contrast between the greenish-blue color of the white matter and the colorless gray matter. Care should be taken to rinse only briefly in the lithium carbonate solutions (3 to 5 seconds) since the final most delicate differentiation occurs in 70% alcohol.
10. Wash thoroughly in distilled water.
11. Stain from 1 to 2 minutes in Solution B (which should be carefully warmed before using).
12. Wash briefly in distilled water. (This step is necessary only when using the Coleman and Bell product.)
13. Differentiate in several changes of 95% alcohol.
14. Clear in a xylol-terpineol mixture.
15. Clear in xylol and mount.



6. Begin differentiation by placing immersion in 10% alcohol

aqueous lithium carbonate solution.

7. Continue differentiation in several changes of 70% alcohol until gray and white matter can be distinguished.

Care should be taken not to overdifferentiate.

8. Wash in distilled water.

9. Finish the differentiation by briefly rinsing in 0.5% lithium carbonate and then putting through several changes of 70% alcohol until there is a sharp contrast between the greenish-blue color of the white matter and the colorless gray matter. Care should be taken to rinse only briefly in the lithium carbonate solution (3 to 5 seconds) since the final most delicate differentiation occurs in 70% alcohol.

10. Wash thoroughly in distilled water.

11. Stain from 1 to 2 minutes in solution B which should be carefully warmed before using.

12. Wash briefly in distilled water. (This step is necessary only when using the Gollner and Hall product.)

13. Differentiate in several changes of 95% alcohol.

14. Clear in a xylol-terpineol mixture.

15. Clear in xylol and mount.



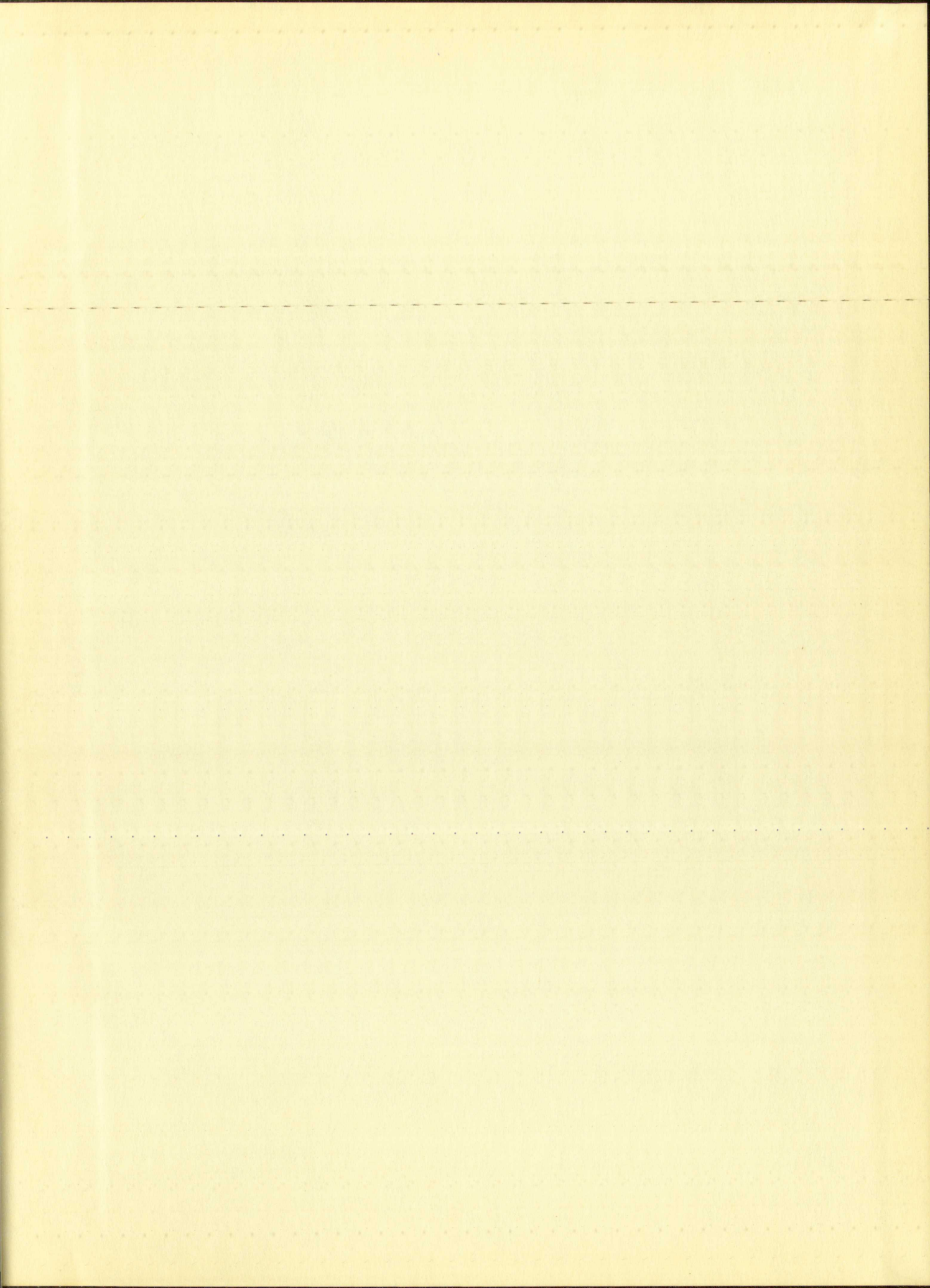




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